Localization and Network of Coma-Causing Brainstem Lesions: Evidence for a Human Consciousness Network

Abstract

Focal brainstem lesions can disrupt arousal and cause coma, yet the exact location of the brainstem region critical to arousal and its associated network are unknown. First, we compare brainstem lesions between 12 patients with coma and 24 patients without coma to identify a region specific to coma-causing lesions. Second, we determine the network connectivity of this brainstem region and each individual coma-causing lesion using resting state functional connectivity MRI data acquired from 98 healthy subjects. Third, we evaluate the functional connectivity of this network in patients with disorders of consciousness (51 patients versus 21 controls). These analyses reveal a small, coma-specific region in the left pontine tegmentum, near the medial parabrachial nucleus. This brainstem region, and each individual coma-causing lesion, is functionally connected to the left agranular, anterior insula (AI), and pregenual anterior cingulate cortex (pACC). These cortical sites align poorly with previously defined functional networks but match the distribution of von Economo neurons (VENs). Finally, functional connectivity between AI and pACC is disrupted in patients with impaired awareness. These results show that coma-causing lesions overlap a specific brainstem location that is functionally connected to specific cortical areas, defining a unique human brain network. The brainstem node, if lesioned, disrupts arousal while the VEN-containing cortical nodes exhibit abnormalities in patients with disrupted awareness. We propose that this network may represent a neuroanatomical substrate linking arousal and awareness, the two principal components of human consciousness.

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Glossary

AI = anterior insula

ARAS = ascending reticular activating system

EEG = electroencephalogram

MCS = minimally conscious state

MRI = magnetic resonance imaging

pACC = pregenual anterior cingulate cortex

PBN = parabrachial nucleus

REM = rapid eye movement

rs-fcMRI = resting state functional connectivity magnetic resonance imaging

VEN = von Economo Neuron

VS/UWS = vegetative state/unresponsive wakefulness syndrome

Introduction

Though fundamental to cognition and behavior, consciousness remains an elusive function of the human brain. The mere endeavor to define consciousness, let alone to identify its neurobiological basis, has been pursued by centuries of philosophy and science. Understanding how the brain generates consciousness remains one of the greatest challenges of modern science, and one with profound implications for both basic neuroscience and clinical care.

Consciousness is thought to consist of two interrelated but conceptually distinct parts: arousal (i.e., wakefulness) and awareness (i.e.,the content of one's experience)[1,2]. Awareness requires arousal, in that one must be awake in order to be aware[1,2] (though dreaming during rapid eye movement [REM] sleep might appear to violate this relationship, REM sleep in fact shares many neuronal characteristics with wakefulness[3]). However, one can be awake without being aware, as exemplified by the vegetative state/unresponsive wakefulness syndrome, in which patients maintain sleep-wake cycles without demonstrating awareness of themselves or their environments[4]. The neuroanatomical substrates supporting these components of consciousness, and supporting their integration, remain unclear. However, arousal is thought to depend predominantly upon the brainstem, whereas awareness is thought to depend more upon the cerebral cortex[1].

The neurobiology of arousal

The importance of the brainstem in sustaining arousal has been appreciated for several decades. This topic was first formally studied in 1935, when a Belgian neurophysiologist Frederic Bremer investigated how different brainstem transections affected arousal in cats[5]. He found that, whereas transections between the medulla and spinal cord did not disrupt arousal, transections between the superior and inferior colliculus – at the level of the midbrain – produced behavioral unresponsiveness and a pattern of electrical discharges from the cortex indicative of slow-wave sleep, as measured with an electroencephalogram (EEG). In 1949, this brainstem system was termed the ascending reticular activating system (ARAS) by neurobiologists Moruzzi and Magoun[6], who demonstrated that electrical stimulation of the midbrain tegmentum

produced EEG patterns resembling wakefulness in anesthetized cats, and that large electrolytic lesions of the midbrain tegmentum suppressed cortical arousal and produced behavioral unresponsiveness[6,7]. The ARAS was termed as such based on the notion that neurons from a broad "reticulum" (i.e., net-like distribution) of neurons "ascended" from the brainstem to the cortex to produce an "activating," or arousing, effect[8]. Subsequent studies in animal models further refined the brainstem region important for arousal, demonstrating that lesions below the level of the mid-pons did not impair arousal, while lesions between the rostral pons and midbrain caused behavioral unresponsiveness and EEG patterns characteristic of sleep[2]. Thus, it became clear that the tegmentum between the rostral pons and caudal midbrain – also termed the mesopontine tegmentum – contained neural structures critical to sustaining arousal[2].

Further investigation of the ARAS revealed that this region of the mesopontine tegmentum was in fact not an amorphous "reticulum," but rather a series of discrete brainstem nuclei, each contributing to arousal through distinct neurotransmitter systems (for this reason, alterative names, such as the "ascending arousal system," are often considered more appropriate than "ARAS"[2]). For example, the mesopontine tegmentum includes the pedunculopontine and laterodorsal tegmental nuclei, whose cholinergic and other neuronal subtypes project through the paramedian midbrain reticular formation to thalamic nuclei[9]. Their thalamic targets include the midline/intralaminar nuclei, which project more diffusely to the cerebral cortex[10], and the reticular nucleus, which is thought to coordinate synchrony of the cortical activity observed on EEG[11]. Activity of some neurons within the pedunculopontine and laterodorsal tegmental nuclei also varies with different behavioral states[12]. During wakefulness, neurons in these nuclei fire rapidly, and during slow-wave sleep, they fire more slowly; moreover, during REM sleep (a state resembling wakefulness on EEG), these neurons fire rapidly again[13,14].

In addition to these cholinergic nuclei, there are at least three monoaminergic nuclei also contained within the mesopontine tegmentum with ascending influence on the cortex. The locus coeruleus, located in the pontine tegmentum, synthesizes and distributes norepinephrine diffusely throughout the cerebral cortex[15]. Neurons in the locus coeruleus fire more rapidly during states of wakefulness, and particular in response to highly arousing stimuli[16]. Their firing slows during slow-wave sleep, and

they become silent during REM sleep[16]. Optogenetic stimulation of the locus coeruleus produces immediate arousal from sleep in mice, extends periods of wakefulness and increases locomotion[17]. The dorsal raphe nucleus, extending from the midbrain to the pons, synthesizes and distributes serotonin throughout the cerebral cortex, and has been implicated in the stress response[18]. Neurons from the ventral tegmental area, located in the midbrain, synthesize and distribute dopamine to select prefrontal and limbic regions of cortex[19]. Some neurons in this region are most active during wakefulness, slowing during slow-wave and REM sleep[20].

Given the associations between their activity patterns and states of sleep and arousal, neurons from these cholinergic and monoaminergic nuclei were thought to play an important role in sustaining wakefulness[2]. However, studies later revealed that, though these nuclei may promote arousal, they are not strictly necessary for it: eliminating these cell groups does not significantly disrupt sleep-wake states or cortical EEG[21–24].

In contrast, recent studies have pointed to a region in or near the medial parabrachial nucleus (PBN) as necessary for arousal. The PBN is a complex of neurons spanning the pons-midbrain junction and surrounding the superior cerebellar peduncle in the dorsolateral pontine tegmentum[25]. Most PBN neurons are glutamatergic[26,27], and some project to subcortical regions implicated in arousal, such as the thalamus, hypothalamus, basal forebrain, amygdala, and the bed nucleus of the stria terminalis[25,28,29]. Studies in mice and rats have shown that the PBN also projects directly to two cortical areas: the insular cortex and prefrontal cortex[29,30]. In 2001, Devor and colleagues identified a region in or near the rostral margin of the PBN where microinjections of pentobarbital (a GABA-A receptor anesthetic) produced deep and reversible anesthesia in rats[31]. In 2011, Fuller and colleagues demonstrated that ablating the region of the medial PBN in rats abruptly caused coma: the rats became behaviorally unresponsive, and exhibited EEG patterns indicative of coma (power in the <1 Hz range)[22]. In 2013, Kaur and colleagues demonstrated that deleting the vesicular glutamate transporter type 2 (*Vglut2*) gene from neurons in the PBN, thereby hampering glutamate release from PBN neurons, also diminished arousal in mice[27]. In addition,

studies in cats have shown that neurons in the parabrachial region exhibit selective activity during wakefulness[32,33].

The neurobiology of arousal in humans

As these studies of arousal have been largely conducted in animal models, the brainstem structures supporting arousal in humans have remained poorly defined. Traumatic injury of the pons and midbrain is associated with the loss of consciousness in humans[34–40]. In 2003, Parvizi and Damasio investigated which human brainstem region is critical to arousal by studying lesions that caused coma[41]. Coma represents the total loss of arousal, and therefore also awareness, and can be caused by metabolic derangement, extensive injury to both cerebral hemispheres, and/or injury of the brainstem[2]. In rare instances, such brainstem injury is due to small, focal lesions. Such lesions can lend insight into the neuroanatomical regions that comprise the human ARAS, and therefore are necessary for human arousal. Recognizing this, Parvizi and Damasio managed to collect 9 cases of coma caused by focal brainstem lesions[41]. After overlapping these lesions in a normalized brainstem template, they observed that 8 of the 9 lesions involved the rostral pontine tegmentum, suggesting that this region is critical to arousal in humans, just as it is in experimental animals.

Though Parvizi and Damasio's study improved our understanding of the brainstem region important for human arousal, the exact localization of this region has remained unclear. The region of the pontine tegmentum identified by their lesion overlap was in the midline, which did not correspond to the more lateral brainstem regions implicated in arousal in experimental animals. There were methodological limitations that may have hampered the precision of localization in their study. First, while cases of coma caused by focal brainstem lesions are quite rare, 9 cases remains a small sample size. Second, it is unclear whether all patients included in this retrospective study were strictly comatose; several were only unresponsive for ~2 hours, or were capable of behaviors (e.g., "odd muttering sounds") inconsistent with coma, suggestive instead of the vegetative state/unresponsive wakefulness syndrome. Including such cases may have clouded the localization. Third, though Parvizi and Damasio did report examining a set of control lesions – brainstem lesions that did not cause disorders of consciousness

– such lesions were not formally incorporated into their analysis. They mention that control lesions tended to occur in the anterior brainstem, but also acknowledge that these lesions sometimes involved the tegmentum. Accounting for these lesions in their analysis – for example, by subtracting out regions that, when lesioned, did not disrupt consciousness – may have further refined the brainstem region(s) truly critical to arousal.

Moreover, though Parvizi and Damasio's study helped to identify the brainstem area important for human arousal, the brainstem does not maintain arousal in isolation. Rather, as indicated by the work in experimental animals, the brainstem region maintains human arousal through ascending projections to additional brain regions[6,7,22,42]. As previously mentioned, several brain regions have been implicated in arousal, including the thalamus, hypothalamus, basal forebrain, amygdala, and bed nucleus of the stria terminalis[8,10,22,25,42,43]. However, the extent to which these areas are necessary for arousal, and/or communicate with brainstem structures necessary for arousal, remains unclear in humans. Edlow and colleagues recently investigated this question using diffusion tractography, a technique for imaging fiber tracts[44]. By measuring the directionality of water diffusion across axonal bundles, this imaging modality enabled the investigators to identify tracts communicating between brainstem nuclei potentially important for arousal (e.g., PBN, locus coeruleus, dorsal raphe, pedunculopontine nulcues, ventral tegmental area) and other brain regions. With this method, they found tracts between these brainstem nuclei and other regions implicated in arousal, such as the thalamus, hypothalamus and basal forebrain. However, this approach remains limited. First, it remains unclear which nucleus or nuclei are critical to human arousal. Second, it is unclear which tracts between them and their several targets are functionally relevant to arousal. Lastly, this tract-based imaging methodology is limited in detecting tracts with small numbers of axons, circuitous courses, crossing fibers, or polysynaptic connections between regions[45].

The neurobiology of awareness

As compared to arousal, the neurobiology of awareness has remained an especially challenging topic of investigation. Due in part to its inherently subjective

nature, and the absence of any clearly measurable features, awareness is difficult to operationalize and study in a rigorous fashion. Nonetheless, whereas arousal is thought to depend upon the brainstem, awareness is thought to depend more upon the cerebral cortex, such that information is integrated across sensory and cognitive modalities into a unitary conscious experience[1]. However, which cortical regions, if any, are particularly critical to supporting awareness remain largely unknown.

One approach to investigating the cortical substrates of awareness is to study the regions that are dysfunctional in patients with disrupted awareness. Patients in the vegetative state/unresponsive wakefulness syndrome, or in the minimally conscious state, maintain arousal but have impaired awareness. While metabolism and structural integrity of the brainstem is intact in these patients, cortical metabolism is reduced by 50-60%[46,47]. Some have found particular dysfunction of certain cortical regions, though results have been mixed; a meta-analysis of functional neuroimaging from patients with disorders of consciousness found reduced activity in a variety of regions, including the cingulate cortex, precuneus, frontal cortex, and medial temporal cortex, as well as the thalamus[48]. Numerous studies have also investigated patterns of disrupted connectivity between brain regions in patients with disorders of consciousness[49–58], and while also mixed, have often demonstrated dysfunction and impaired connectivity of the default mode network – a network active in the absence of goal-directed tasks, and associated with self-reflection and internal cognition[59,60].

The cortical substrates of awareness have also been investigated in healthy individuals, through a variety of methods aimed at dissociating sensory perception from awareness of stimuli[47,61]. For example, in ‘visual masking’, a visual stimulus (the “target”) is presented briefly, followed by a second visual stimulus (the “mask”)[62]; by adjusting the duration of the target presentation, experimenters can modulate whether stimuli are consciously or unconsciously perceived. Similarly, in ‘attentional blink’, a target visual stimulus is often not consciously perceived if presented within a few hundred milliseconds after an initial visual stimulus[63]. In ‘binocular rivalry’, different visual stimuli are presented to each eye, and only one can be consciously perceived at a time[64]. Investigators have used such methods to compare which cortical regions are active not only during exposure to visual stimuli, but during awareness of visual stimuli,

through neuroimaging techniques and even neuronal recordings in non-human primates: while primary visual cortex tends to be active during exposure to all visual stimuli, several other regions become selectively active during awareness of stimuli, such as the anterior insula, anterior cingulate, inferior parietal lobe, lateral frontal cortex, and inferior temporal cortex[47,61,65–67].

Given inconsistency in the cortical regions implicated in awareness, others have explored temporal, rather than spatial, mechanisms of awareness[47]. While both consciously and unconsciously perceived visual stimuli elicit early event-related potentials on EEG associated with sensory processing, consciously perceived stimuli elicit a prolonged wave of activity[68,69], leading some to propose that awareness depends upon sustained neuronal activity in response to stimuli. Others have suggested that while all stimuli elicit an initial wave of neuronal activity (i.e., a “feedforward sweep”), awareness only occurs after a second wave is returned from higher to lower cortical areas (i.e., a “reentrant” or “recursive” wave)[70]. Yet others have theorized that awareness requires synchronous oscillations in activity across the cortex to bind various sensations into a unitary sensory experience[47,71].

In addition, some have explored the possibility that certain cell types in the brain play a role in supporting awareness. In 1918, an Austrian neuroanatomist, Constantin von Economo, discovered peculiar neurons in the anterior insula and anterior cingulate of the human brain, which he later described as “Stäbzellen” (rod cells) and “Korkzieherzellen” (corkscrew cells)[72–74]. These spindle-shaped cells have since been renamed von Economo Neurons (VENs)[72,75,76], and have been found in the anterior insula and anterior cingulate of particular mammals, including other great apes[75,77], whales[78], dolphins[79] and elephants[79]. Based on their large size and narrow dendritic arborization spanning the cortical layers, VENs have been proposed to rapidly integrate and transmit information across large brains[77]. Intriguingly, it has also been noticed that species with VENs in the anterior insula and anterior cingulate are generally capable of passing the mirror test[80–82]. (In the mirror test, a mark is typically placed on the head of the animal, and the animal is presented with a mirror; if the animal uses its reflection to locate the mark, and proceeds to touch the mark on its own head, it is thought capable of a degree of self-recognition, or self-awareness[80].) In addition, selective degeneration

of VENs has been observed in frontotemporal dementia, a condition in which cognition and self-regulation are impaired[76]. Thus, though the precise function of these neurons remains unknown, it has been speculated that they play a role in some form of self-awareness[76,83].

As reviewed, many diverse neuroanatomical substrates and mechanisms have been proposed to support human awareness. Yet, the results of such investigations have been mixed, and thus the neurobiology of awareness remains poorly defined. In comparison, the neurobiology of arousal has been more rigorously studied and better characterized, likely in part because arousal can be more objectively measured and more easily operationalized. Thus, arousal serves as a more tractable entry point in investigating the neurobiology of consciousness.

Purpose of inquiry

This paper aims to elucidate the neuroanatomical substrates of human arousal through several complementary approaches. First, we aim to identify the brainstem region critical for human arousal. Drawing from but expanding on the methodology of Parvizi and Damasio, we conduct the largest lesion overlap analysis of coma-causing brainstem lesions to date. We improve the specificity of this analysis by using strict clinical criteria for coma and incorporating control lesions that do not impair arousal.

The second aim is to identify the forebrain regions associated with this brainstem region, which together may form a brain network critical for arousal. Resting state functional connectivity magnetic resonance imaging (rs-fcMRI) is a modality well suited for this aim. Rs-fcMRI identifies spatially disparate regions of the brain that exhibit synchronous (i.e., correlated) fluctuations in the blood-oxygen-level dependent fMRI signal[84]. Such regions with synchronous fluctuations in activity are considered to be 'functionally connected'[84]. In comparison to structural tract imaging, rs-fcMRI is better able to identify functionally connected regions across polysynaptic and circuitous connections. Rs-fcMRI has already proven useful in identifying a variety of functionally important brain networks[85], including networks functionally connected to regions of the brainstem[86,87].

To identify the network associated with the brainstem region critical to arousal, we will use rs-fcMRI in a non-traditional way. Rather than using rs-fcMRI from patients with disorders of consciousness, as numerous studies have done before[49–58], we will first investigate this network in the healthy brain. We will input the brainstem region critical to arousal into a rs-fcMRI dataset acquired from healthy individuals, in order to identify which regions are normally functionally connected to this brainstem region. Existing evidence suggests that such an approach may be useful; inputting the locations of lesions into a normative rs-fcMRI dataset can predict which networks will be affected by those lesions[88].

Our third aim is an exploratory one: once we have identified the brainstem region critical to arousal and its associated network, we will conduct additional analyses to investigate the properties of this network, including its connectivity pattern, laterality and relation to previously identified networks. We will also use histological analyses to examine this network's neuronal composition.

Our final aim is to apply these findings to patients by assessing the relationship between connectivity of this identified network and disorders of consciousness. Based on the rs-fcMRI studies previously conducted in such patients, disorders of consciousness have frequently been attributed to dysfunction of the default mode network[49,51,53,55,56,58,89–91]. Thus, we will compare how connectivity of our identified network compares to that of the default mode network in a cohort of patients with disorders of consciousness.

This work has significant implications for basic and clinical neuroscience. Identifying the brainstem region critical to arousal and its associated network may help to elucidate the neuroanatomical underpinnings of human consciousness, one of the most fundamental yet enigmatic functions of the human brain. Understanding the brainstem region affected by coma-causing lesions, as well as the network disrupted in disorders of consciousness, would improve our understanding of these disorders, some of the most devastating in neurology. A neurobiological characterization of these disorders, in turn, may help to advance diagnosis and therapy. Moreover, as both invasive[92] and noninvasive[93] methods of brain stimulation have been investigated for

treating disorders of consciousness, a better characterization of these neuroanatomical substrates may lead to more effective targets for therapeutic brain stimulation.

Materials and Methods

Lesion acquisition

We collected coma-causing lesions from cases either encountered by the authors or previously reported in the literature[41,94–97]. We defined coma by the absence of wakefulness, auditory/visual response, communication, spontaneous movement, or response to noxious stimuli, according to accepted guidelines[4]. We considered only coma cases attributable to an acute-onset brainstem lesion, where the lesion was clearly visualized through brain imaging or autopsy. Exclusion criteria for coma cases included: brief episodes of unresponsiveness (<3 hours), extensive injury elsewhere in the brain that could account for coma, concurrent toxic-metabolic disturbances, seizure, extensive distortion of brainstem anatomy, or poorly delineated lesion boundaries. We identified literature cases through a systematic search of www.pubmed.gov, with search terms of “coma,” “brainstem,” “human,” and “lesion.” Some cases diagnosed as coma in the literature did not meet our criteria and were thus excluded (e.g., cases S.A., T.G., H.R., L.P. and V.S. from Parvizi and Damasio, 2003).

Inclusion criteria for control cases required an acute-onset brainstem lesion that did not impair arousal or cause a disorder of consciousness. Exclusion criteria for control lesions included extensive distortion of brainstem anatomy, poorly delineated lesion boundaries, or lesions limited to the medulla (as all coma lesions involved the pons or midbrain). We selected controls from local cases and previous reports of brainstem lesions causing motor deficits, using such search terms as “lesion,” “pons,” “midbrain,” “motor,” and “locked-in”[95–97]. The Partners Institutional Review Board approved this study.

Lesion analysis

Using anatomical landmarks as a guide, we manually reproduced the two-dimensional contour from every lesion onto comparable slices of a template brain

(MNI152 atlas, 1 mm x 1 mm x 1 mm) using FSL (http://fsl.fmrib.ox.ac.uk/fsldownloads/). Two investigators jointly evaluated each reproduction to ensure accuracy. Next, we transformed the lesions into three dimensions by viewing the two-dimensional reproductions in orthogonal planes and connecting the lesion edges. See Supplementary Fig. 1B-F for an overview of the lesion processing method.

To identify the coma-specific brainstem region (an area maximally involved by coma lesions and minimally involved by control lesions), we separately overlapped all coma and control lesions, and subtracted the control lesions from the coma lesions[98]. We also conducted voxel-based lesion-symptom mapping to compare coma and control lesions, using the Liebermeister approach for binomial data[99]. We thresholded the Z-score results at 3.54 ($p < 0.05$, correcting for false discovery rate). For display purposes, results were overlaid on a high-resolution template brain (0.5 mm x 0.5 mm x 0.5 mm) using FSL and MRIcron (http://www.mccauslandcenter.sc.edu/mricro/mricron/).

To identify the brainstem nuclei that approximate the coma-specific region, we analyzed Nissl cytoarchitecture in thionin-stained brainstem sections prepared in a previous study[100]. Using a flatbed photo scanner (Epson V700), we acquired 1200 dpi images of 20 slices (50 μm-thick, spaced 1 mm apart) between the mid-pons and caudal midbrain. We selected the rostral pontine slice that best approximated the axial MRI level containing the coma-specific region, and then labeled brainstem structures atop it in Adobe Illustrator while referring to the section in the microscope.

Network analysis

We used rs-fcMRI to investigate the brain network associated with the coma-specific brainstem region, as well as with each individual lesion[84,88]. For this analysis we utilized a normative rs-fcMRI connectome dataset acquired from 98 right-handed, healthy subjects (48 males, ages 22 ± 3.2 years); dataset details have been reported previously[88,101].

We subsampled the coma-specific region to 2 mm x 2 mm x 2 mm resolution (to match the resolution of the rs-fcMRI data), binarized it, and entered it as a seed within the rs-fcMRI connectome dataset. We correlated the BOLD signal time course within

this region to that of every other brain voxel, for each of the 98 subjects. We converted each voxel correlation r value to a z-score via Fisher's r-to-z transformation, and then to a t-score through a random effects one-sample t-test across all 98 participants. We thresholded the resulting network at a t-value of 4.25 (indicating regions of correlation at $p < 0.00005$, uncorrected[101]), and restricted results to a brain mask (MNI152 brain 2 mm x 2 mm x 2 mm, available in FSL). We identified functionally connected nodes of at least 100 voxels, or 800 mm^3, with a cluster identification algorithm in FSL. Because the global signal has been associated with arousal[102], we re-assessed functional connectivity of the coma-specific region without global signal regression (threshold $t > 10.5$, $p < 0.00001$, uncorrected). For display purposes, rs-fcMRI results (2 mm x 2 mm x 2 mm resolution) were overlaid on a high resolution template brain (0.5 mm x 0.5 mm x 0.5 mm resolution) using FSL and MRIcron.

We next identified nodes functionally connected to each individual lesion using the recently described lesion network analysis[88]. We entered each lesion (coma or control) as a seed in the rs-fcMRI dataset, thresholding each resulting network at a t-value of 4.25 and binarizing above this value[88]. We identified regions functionally connected to all coma lesions, as well as regions significantly more connected to coma lesions than control lesions (voxel-wise t-test, threshold $t > 2.04$ and < -2.04, $p < 0.05$, DF = 34, uncorrected). The voxel-wise t-test results comparing coma to control lesions were masked to show only voxels that were also significantly connected to coma lesions. We repeated this voxel-wise t-test replacing each lesion with a sphere (radius 5 mm) at the lesion's center of gravity to eliminate any confounding effect of size difference between coma and control lesions.

Network refinement

We conducted a variety of sub-analyses to further investigate the anatomy of the identified network. First, we used partial correlation to assess the directness of connectivity between network nodes[103]. Correlations between pairs of nodes were computed while partialling out the third, both on a regional and voxel-wise basis. Analyses were computed using both weighted and binarized regions of interest, to ensure that this did not influence the results.

Second, we assessed the connectivity of the cortical nodes to the pons by entering each cortical node as a seed in a rs-fcMRI analysis. To identify peak correlations in the pons, results were thresholded ($t > 9$, $p < 0.00001$, uncorrected) and masked using an anatomical template of the pons (Harvard-Oxford Subcortical Structural Atlas, threshold 35).

Third, as our coma-specific brainstem region was left-lateralized, we investigated the impact of this laterality on our identified cortical network. We flipped our coma-specific region to the right, repeated our rs-fcMRI analysis, and compared the results to the original results obtained using the left-sided brainstem node.

Von Economo neuron plots and imaging

The cortical regions identified by our network analysis resembled previously reported locations of von Economo Neurons (VENs). To assess the correspondence between the identified network nodes and the distribution of VENs in the human brain, we developed a brain-wide mask of the VEN distribution. We imaged a post-mortem brain with MRI, then sliced that brain into 50 μm thick, coronal frozen sections. We Nissl-stained the sections on glass slides. Based on established morphological criteria[72], we identified and plotted VENs (1 in 10 sections, or 0.5 mm resolution) with a computer-aided plotting system (MD Plot, Accustage). We outlined the plots and superimposed them onto the MR images, creating a binarized brain-wide mask. Using anatomical landmarks (e.g., sulci and gyri), we manually transformed the mask into MNI space.

To illustrate the relative restriction of VENs to the agranular region of the insula, we analyzed thionin-stained (Nissl) human brain tissue sections prepared in a previous study[104]. We selected for further analysis a brain slice with a section angle best approximating the standard coronal MRI plane. Using a flatbed photo scanner (Epson V700), we acquired whole-slide images from a series of insular sections (50 μm, spaced 1 mm apart). For plotting VENs, we chose the section that most closely corresponded to the center of the insular node identified in our network analysis. We plotted brain structures at 50x and VENs at 10x in Neurolucida (MBF Bioscience) using a Zeiss light microscope with a motorized stage. We imported the resulting Neurolucida plot into

Adobe Illustrator, overlaid it on the whole-slide image and re-drew both as filled, smooth contours and red dots.

We quantitatively evaluated the correspondence of the identified cortical nodes to the brain-wide VEN distribution, as well as to previously defined resting state networks, such as the salience and default mode networks[85,105]. For this analysis, we binarized the identified cortical nodes and computed their overlap with binarized masks of the brain-wide VEN distribution and of resting state networks previously defined by two different techniques[85,106]. For each node, we expressed overlap as: (number of voxels within both the node and mask) / (number of voxels within the node).

Subcortical network analyses

At our relatively strict statistical threshold ($t > 4.25$), the primary rs-fcMRI analysis of our coma-specific brainstem region failed to identify functional connectivity to several subcortical sites previously implicated in arousal[8,22,25,42]. We therefore relaxed statistical thresholds and examined connectivity specifically to some of these structures, such as the thalamus ($t > 3.75$, $p < 0.0005$, uncorrected) as well as the hypothalamus, basal forebrain, amygdala, and bed nucleus of the stria terminalis ($t > 2$, $p < 0.05$, uncorrected). Anatomical masks were derived from the MNI Structural Atlas available in FSL (thresholded at 35), or from histology/high-resolution imaging[43,107,108]. Each subcortical structure was also tested for connectivity to individual coma lesions versus control lesions as described above.

Network connectivity in disorders of consciousness

To assess connectivity of the identified network in disorders of consciousness, we used an independent rs-fcMRI dataset acquired from 26 patients in a minimally conscious state (MCS), 19 patients in a vegetative state/unresponsive wakefulness syndrome (VS/UWS), 6 patients in coma (with etiologies other than focal brainstem lesion), and 21 healthy controls (dataset details reported elsewhere[54]). We restricted our analysis to the cortical nodes of this network, given poor signal-to-noise ratio in the brainstem in this dataset and to facilitate direct comparison with other networks. We compared connectivity between the two cortical nodes of our network to connectivity between nodes of the default mode network (medial prefrontal cortex and posterior

cingulate cortex) and motor network (hand area of the left and right motor cortex). We represented each node with a sphere (radius 5 mm) placed at the node's center of gravity; we obtained node coordinates for the default mode[85] and motor network[109] from prior work. We then compared network connectivity between healthy controls, patients with coma, and patients with disorders of consciousness characterized by impaired awareness (MCS and VS/UWS). We collapsed across MCS and VS/UWS in our main analysis to maximize statistical power and because connectivity between our cortical nodes did not significantly differ between these conditions. To standardize across networks, we expressed inter-node connectivity as a ratio of connectivity in healthy controls. We used t-tests to identify whether network connectivity differed from zero, differed between groups, or differed between networks.

Results

Lesion analysis

We collected 36 focal brainstem lesions from patients encountered locally or described in the literature[41,94–97]. Twelve lesions caused coma (7 local cases, 5 literature cases; mean age 57.3±16.5; mean lesion volume 3540±2988 mm^3), while 24 control lesions did not (8 local cases, 16 literature cases; mean age 58.1±12.8; mean lesion volume 2364±2644 mm^3). There was no significant difference in volume between the coma and control lesions ($p = 0.26$). See Supplementary Table 1 and Supplementary Fig. 1A for additional case details.

Coma-causing lesions maximally overlapped in the pontine tegmentum (10 of 12 cases; Fig. 1A). In contrast, control lesions primarily involved the pontine base (11 of 24; Fig. 1B). Subtracting control lesions from the coma lesions, or performing voxel-based lesion-symptom mapping, revealed a 2 mm^3 'coma-specific region,' ($p < 0.05$, corrected for false discovery rate). This coma-specific region was lateralized to the left pontine tegmentum, approximating the medial PBN (Fig, 1C, D, E). Ten of 12 coma lesions, but only the periphery of 1 of 24 control lesions, involved this region (Supplementary Fig. 2A). The 2 coma lesions that spared this region involved the midbrain immediately rostral to it, potentially disrupting ascending tracts (Supplementary

Fig. 2B, C, 3A). Several coma-causing lesions involved this left-lateralized region alone, without extending into the right pontine tegmentum or midline midbrain.

Network analysis

To identify brain regions in the network of the coma-specific region, we performed a resting state functional connectivity analysis using MRI data from a large cohort of healthy subjects. This analysis revealed two nodes functionally connected to our brainstem region: one node was located in the left agranular, anterior insula (AI; in a subregion also known as frontoinsular cortex), and the second was located in the pregenual anterior cingulate cortex (pACC; Fig. 2, Supplementary Fig. 3B, C, Supplementary Table 2). These findings were similar with or without regression of the global signal, which has been associated with arousal[102] (Supplementary Fig. 4). Voxels within both nodes were functionally connected to all 12 coma lesions (Fig. 2C), and were significantly more connected to coma lesions than to control lesions ($p < 0.05$; Fig. 2D), even after lesion volumes were normalized (Supplementary Fig. 5).

These nodes do not correspond well to any cortical network previously defined by rs-fcMRI analysis[85,105]: the AI node straddles the salience network, limbic network and default mode network, while the pACC node falls mostly within the default mode network. Rather, the AI and pACC nodes are histologically unique as the two primary sites of von Economo neurons (VENs), spindle-shaped neurons found in a select group of mammalian species (see discussion). Microscopic plotting of VENs in a human brain revealed that the currently-identified AI and pACC nodes correspond more closely to the VEN distribution than to any major resting state network (Fig. 3, Fig. 4, Supplementary Fig. 6).

In experimental animals, neurons in the brainstem region corresponding to our coma-specific region project to several subcortical sites implicated in arousal, including the thalamus, hypothalamus, basal forebrain, central nucleus of the amygdala, and bed nucleus of the stria terminalis[8,22,25,42]. None of these regions appeared in our initial analysis ($t > 4.25$), so we relaxed our statistical threshold in these regions. Doing so revealed connectivity to the midline and lateral thalamus, posterior hypothalamus and basal forebrain (Supplementary Fig. 7); there was virtually no connectivity to the

amygdala or bed nucleus of the stria terminalis. However, unlike the AI and pACC nodes, none of these regions were significantly more functionally connected to coma lesions than control lesions.

We conducted additional analyses to investigate the network organization between the coma-specific brainstem region, AI, and pACC. First, we used partial correlations to assess the directness of connectivity between each node[103]. Stronger correlations were observed between the brainstem node and AI ($r = 0.15$) and between the AI and pACC ($r = 0.26$), than between the brainstem node and pACC ($r = 0.06$). This pattern persisted when using unweighted (i.e., binarized) seeds (Supplementary Fig. 8).

Second, we investigated the brainstem connectivity of the AI and pACC nodes. The AI node exhibited connectivity to the original brainstem node, and less so to a symmetric region of the contralateral pontine tegmentum (Fig. 5A). In contrast, the pACC node exhibited connectivity to the midline pontine base, not the tegmentum (Fig. 5B). These findings suggest more direct connectivity between the brainstem node and AI, and between the AI and pACC within this network.

Third, we investigated the laterality of our findings by flipping the brainstem node to the right and re-running the connectivity analysis. The left AI and pACC remained the only cortical sites with significant connectivity to the right-flipped brainstem node. However, compared to the network analysis of the original left-sided brainstem node, connectivity was weaker: the volumes of significant connectivity were smaller (AI 2.0 versus 3.4 cm^3; pACC 1.6 versus 5.6 cm^3) and mean t-scores were reduced (AI 5.43 versus 5.89; pACC 4.75 versus 4.85). The stronger connectivity of the left-sided brainstem node and the left-lateralization of the AI node, even when defined by the flipped right-sided brainstem node, further support a left-lateralization of the identified network.

Network connectivity in disorders of consciousness

Next, we investigated AI-pACC cortical connectivity in patients with disorders of consciousness using rs-fcMRI[54]. We compared AI-pACC connectivity with that of the default mode network, a network previously implicated in disorders of consciousness[49,51,53,55,56,58,89–91], and the motor network, which served as a control. In

comatose patients, connectivity was absent (not significantly different from zero) across all three networks. In patients with disrupted awareness (MCS or VS/UWS), connectivity was reduced in all networks ($p < 0.001$), but only absent between the AI and pACC (Fig. 6). The reduction in AI-pACC connectivity in this group exceeded that of the other networks ($p < 0.05$). Splitting MCS and VS/UWS into separate groups, AI-pACC connectivity was absent in both groups, default mode network connectivity was present in MCS but not VS/UWS, and motor network connectivity was present in both MCS and VS/UWS (Supplementary Fig. 9).

Discussion

Here we identify a human brainstem region causally related to disruption of consciousness and its associated neural network in the healthy brain. First, using coma-causing brainstem lesions we implicate a small region of the left pontine tegmentum, approximating the medial PBN, in human arousal. Second, we show that this brainstem region is functionally connected to the left AI, and secondarily to the pACC, the primary sites of VENs. Finally, we show that patients with disorders of consciousness characterized by impaired awareness exhibit a connectivity deficit between the AI and pACC.

Neuroanatomical substrates for human arousal

Disrupting neurons bilaterally in or near the medial PBN impairs arousal in rodents[22,27,31]. Our findings suggest that a similar brainstem region is necessary for arousal in humans, extending those of Parvizi and Damasio by refining the precise localization of this region. This advance was made possible by a larger group of coma-causing lesions and statistical comparisons to control lesions[99]. Somewhat unexpectedly, in our results this arousal-promoting brainstem region was left-lateralized. Several patients had coma-causing lesions in the left PBN region without extending into the right pontine tegmentum or midbrain. This finding runs counter to conventional wisdom that a lesion must destroy midline or bilateral brainstem tissue to impair consciousness[2,41]. As such, this finding should be interpreted with caution and requires replication. However, there are reasons to think this result may be important. The left

PBN region exhibited stronger connectivity to the identified network than the homologous PBN region on the right, suggesting brainstem connectivity in this region may be asymmetric. Moreover, prior work has shown that the left (but not right) PBN region is more active during wake relative to sleep[110], and that hemorrhages can occur in the right pontine tegmentum without disruption of consciousness[111,112].

Functional connectivity of this brainstem region to the AI and pACC is consistent with direct axonal projections from the PBN to both of these cortical sites (the only direct cortical projections from the PBN)[25,29], and from the AI to ACC[113], previously identified in experimental animals. Diffusion tensor imaging has identified tracts between the AI and ACC in humans as well[114]. Given that the identified brainstem region is critical to arousal, these functionally connected cortical regions may also play a role in arousal, a notion consistent with existing evidence. The pACC and a region including AI are more active during wakefulness[110,115], the AI is overactive in patients with insomnia[116], and lesions of the insula have been associated with fatigue[117]. These regions are linked to situational arousal: the AI becomes more active during anticipatory periods of a gambling task[118], and the AI and ACC, along with the amygdala, are the brain regions most commonly implicated in neuroimaging studies of anxiety[119]. The AI and ACC have both been implicated in autonomic arousal as well: the sympathetic component of heart rate variability and pupillary dilation are correlated with activity in the AI and ACC[120–123], AI activity increases with increased galvanic skin responses[124], and patients with ACC injury show diminished autonomic cardiovascular responses to cognitive effort[122].

Neuroanatomical substrates for human awareness

Though such studies suggest that the AI and ACC are involved in arousal, mounting evidence has also implicated these cortical regions, and particularly the AI, in awareness, or the content of experience. Activity in the AI has been associated with awareness of stimuli across a range of modalities[83,121,125], including pain/temperature (the objective intensity of a painfully hot stimulus is correlated with activity in the posterior insula, but the *subjective* evaluation of the heat is correlated with activity in the AI[83,126]), interoception (AI activity predicts awareness of one's own heartbeat[127], and is associated with sensations such as fullness, bladder distension and thirst[83,128]), auditory

stimuli[129,130], and visual stimuli[130–132]. Injury of the insula has also been associated with altered awareness, such as anosognosia (the unawareness of deficits)[133,134].

Moreover, the AI is the only identified human brain region where electrical disruption has been found to selectively impair conscious awareness[135]. In an epileptic patient undergoing depth electrode evaluation, Koubeissi and colleagues inadvertently found that high-intensity stimulation in the region of the left AI produced immediate unresponsiveness: the patient, though awake, ceased all behaviors, stared blankly, and did not respond to any auditory or visual commands. Immediately after the stimulation was stopped, the patient returned to baseline with no recollection of the events during the stimulation period. It is important to note that the stimulation never produced epileptiform discharges on EEG surface or intracortical leads, so this phenomenon could not be explained as partial-complex seizure activity. Further, the effect was highly focal; no other stimulation sites, including adjacent contacts on the same electrode, elicited the same phenomenon. The investigators were able to render the patient unconscious with high intensity AI stimulation on 10 separate occasions, on 2 separate days of testing. Each time, the patient became entirely unresponsive, occasionally repeating 1 or 2 incomprehensible syllables (further demonstrating that the effects were not purely attributable to motor suppression). We have modeled the spread of current from their electrode contact, relative to the location of the AI node identified here, in Supplementary Fig. 10.

The relationship of the AI to the rest of the brain has also been investigated in the epilepsy literature. Injection of neuroexcitatory agents in a region near the AI in rodents, referred to as the “area tempestas,” reliably induces generalized clonic seizures in rats[136]. Adjacent regions of the insular cortex and claustrum are similarly epileptogenic[137]. In humans with focal or secondarily generalized seizures, a region near the AI has been identified as a common site of origination for epileptic discharges, based on EEG/fMRI and flumazenil binding[138]. The AI’s capacity to trigger seizures may reflect diffuse connectivity with other brain regions, as would be expected of a region that integrates diverse sensory modalities into a unitary conscious experience. Moreover, the capacity of seizures to interrupt awareness may be attributable to dysfunction of this region.

Diffuse connectivity of the AI to the rest of the brain is also suggested by the AI's close proximity to the claustrum[135]. This proximity is evolutionarily conserved; anatomists use the overlying claustrum to define the boundaries of insular cortex across species. The claustrum, a thin sheet of neurons between the external and extreme capsules, projects to and receives input from across the cortex, and thus was proposed to play an integrative role in consciousness by Francis Crick in his later career[139]. While this proposal has yet to be substantiated, it is notable that the AI and claustrum are not only in close proximity, but exchange neuronal projections[140]. It is therefore possible that the capacity of the AI to integrate sensory information across distributed cortices is related to the AI's communication with the nearby claustrum. The anterior-medial extent of the claustrum, which is included within the AI node identified here, may be uniquely important in this capacity.

The ACC has been shown to communicate closely with the AI, as these regions are directly interconnected and frequently co-activate in functional neuroimaging studies[83,121,125,141,142]. Like the AI, the ACC becomes active during awareness of stimuli, albeit less consistently than the AI[83]. In addition, the ACC is thought to monitor and process sensory information to alter behavioral and autonomic function[83,121,141], which is congruent with evidence that information flows predominantly from the AI to ACC[143]. Based largely on functionally neuroimaging data, the ACC has been implicated in allocating attention to external stimuli[144,145], and in processing sensory information to initiate volitional responses[83,141]. These roles have been invoked to explain why lesions of the ACC frequently cause abulia (or in extreme cases, akinetic mutism)[146] and slower responses across cognitive tasks[147], as well as why cingulotomy impairs spontaneous initiation of behavior and intention formation[148]. There are also reports of lesions in the region of the ACC causing loss of consciousness, though such outcomes are rare[146]. In aggregate, this evidence suggests that the ACC plays a role in maintaining conscious responsiveness, perhaps by processing sensory information from the AI.

Our connectivity findings in patients with disorders of consciousness add to existing evidence implicating the AI and pACC in awareness. In our cohort of comatose patients, who have lost both arousal and awareness, cortical networks are globally depressed, consistent with the impaired ARAS function and loss of cortical arousal

characteristic of coma[2]. In contrast, patients with MCS and VS/UWS, who have disrupted awareness but preserved arousal, maintain some network connectivity but have lost connectivity between AI and pACC. These findings suggest that AI-pACC connectivity may play an important role in maintaining awareness, a possibility that warrants further experimental testing.

The brainstem-AI-pACC network

The regions of the AI and pACC identified here do not correspond to any previously identified resting state cortical network. They do, however, overlap nodes of the default mode and salience networks, which may account for the reduced connectivity previously observed in these networks in patients with persistent coma, other disorders of consciousness, and loss of consciousness due to sleep or general anesthesia[49,51–53,55,56,89–91]. Indeed, in deep sleep, diminution of default mode network connectivity is primarily due to decreased connectivity of the ACC node[90]. It is notable that while AI-pACC connectivity is absent in both MCS and VS/UWS, default mode network connectivity is only absent in VS/UWS. Whereas VS/UWS patients lack any signs of conscious awareness, MCS patients retain a small and intermittent level of conscious awareness[4]. Thus, loss of AI-pACC connectivity may sensitively indicate gross perturbations in awareness, while loss of default mode network connectivity may specifically indicate more severe loss of awareness.

Though this cortical network does not correspond to previously defined resting state networks, it does, intriguingly, correspond to the distribution of VENs[72,75,76]. As described earlier, these large, spindle-shaped neurons span the cortical layers, and are found in the AI and ACC of particular mammals, such as humans[72], other great apes[75,77], whales[78], dolphins[79] and elephants[79]. These mammals are generally capable of self-recognition in the mirror test[80–82], and when VENs are present in species incapable of self-recognition, they are often indiscriminately distributed throughout the brain[149]. Moreover, selective degeneration of VENs has been observed in frontotemporal dementia, a condition in which self-regulation is impaired[76]. For these reasons, VENs have been speculated to play a role in some form of self-awareness[76,83].

Our results quantitatively demonstrate that the locations of these neurons closely coincide with the locations of the AI and pACC nodes identified here.

In the network we have identified, the AI node is left-lateralized, even when generated from a right-sided brainstem seed. Although unexpected, the implication of left-lateralized cortical regions in sustaining consciousness is supported by several prior findings in humans: left hemispheric inhibition with intracarotid amobarbital more frequently disrupts consciousness than right-sided injections[150]; impairment of the left cerebral hemisphere (with relative preservation of the right) has been reported in patients with disorders of consciousness[151]; decreased connectivity within the left hemisphere, but not the right, predicts diminished levels of consciousness[152]; seizures that impair consciousness tend to originate in the left hemisphere[153]; some evidence, though mixed, suggests that left-sided strokes are more likely to cause disorders of consciousness[150,154]; and it was the left AI that, when electrically disrupted, eliminated consciousness[135]. Collectively, these results are consistent with the theorized role of the left hemisphere in integrating modular brain functions into a unified conscious experience, as derived from studies in split-brain patients[155].

Although the brainstem node did exhibit connectivity to the thalamus, posterior hypothalamus and basal forebrain – subcortical structures connected to the PBN region and previously implicated in arousal[8,10,22,25,42] – these structures were not specifically associated with coma-causing lesions over control lesions. It is possible that the closer proximity of these structures to the brainstem may have magnified the variance of connectivity, thereby obscuring group differences. However, some of these structures may in fact not be necessary for maintaining consciousness: large thalamic ablations, for instance, cause no impairment in wakefulness in experimental animals[22,156,157]. In addition, it is notable that the AI node is located in the agranular region of insular cortex, which lacks a layer IV, or internal granular layer. Layer IV is the primary target of thalamocortical afferents, and its absence from this region suggests that thalamic information may have relatively little influence over communication between the brainstem, AI node and pACC node.

In light of the evidence provided, it is worth considering whether the brainstem-AI-pACC network identified based on coma-causing lesions may play a role in human consciousness. As discussed, consciousness is comprised of two components: arousal and awareness[1,2]. While arousal is sustained through ascending projections of brainstem structures, awareness is thought to depend more upon the cerebral cortex[1,2,46]. Though these two processes are thought to be interdependent, no neuronal association between these two systems has been established in humans to date. The network defined here links an arousal-promoting brainstem region to cortical regions implicated in both arousal and awareness, providing a neuroanatomical interface between these two fundamental components of human consciousness.

Limitations and suggestions for future work

There are several limitations to the current work, some of which provide important avenues for further investigation. First, due to the relative rarity of brainstem coma, several cases were acquired from a retrospective literature review, creating potential for selection and reporting bias. Prospective validation in an independent and larger cohort may further refine the brainstem region necessary for arousal.

Second, the functional connectivity results, though compelling in aggregate and in the context of prior research, are associational. Interventional studies would help to further establish the causal importance of this network in sustaining consciousness. One potential approach to such investigation, and one with profound clinical relevance, might involve stimulating regions of this network in patients with disorders of consciousness. Brain stimulation in such patients has already been investigated, with mixed results. Deep brain stimulation of the thalamus has occasionally resulted in transiently increased cortical arousal on EEG and improved behavioral responsiveness in patients with disorders of consciousness, but inconsistently and often without improving clinical outcomes[92,158–160]. While outcome heterogeneity may reflect differences in method and patient population[92], it is also possible that the thalamus is not the optimal stimulation site, particularly given recent evidence that the thalamus may not be critical to sustaining consciousness[22]. Deep brain stimulation of the AI, pACC, or even the brainstem node may prove more efficacious (though, as illustrated by

Koubeissi and colleague's case report[135], high intensity stimulation of these regions may be detrimental). More recently, noninvasive methods of brain stimulation have also been tested in patients with disorders of consciousness: in a double-blind, sham-controlled, randomized crossover study, Thibaut and colleagues found that anodal transcranial direct current stimulation of the left dorsolateral prefrontal cortex led to transient improvements in consciousness in patients in MCS[93]. It is interesting to note that the left dorsolateral prefrontal cortex shows weak functional connectivity (less than the AI and pACC) to the parabrachial brainstem node identified here (Supplementary Fig. 11). Thus, while the AI and pACC are too deep for most methods of noninvasive brain stimulation, using transcranial direct current stimulation to target more superficial nodes of this network, such as the dorsolateral prefrontal cortex, may indirectly excite this network. This approach of noninvasively and indirectly stimulating deep regions of a network by targeting superficial nodes has been previously explored, and may hold promise[86].

The importance of this network in sustaining consciousness could also be assessed through lesions of the AI or pACC. However, though naturally occurring lesions of the AI and ACC can disrupt aspects of consciousness (e.g., anosognosia from AI lesions[133,134] or akinetic mutism from ACC lesions[146]), it is noteworthy that lesions of the AI or ACC do not routinely obliterate awareness. In experimental animals, ACC lesions can impair arousal associated with exposure to novel stimuli[24], and AI lesions can suppress fear-related arousal[161], but such lesions are also not typically associated with frank loss of consciousness. Thus, a third limitation of this study is that our findings do not account for the relatively benign impact of lesions to these forebrain nodes on consciousness. There are, however, possible explanations that could be investigated with further research. Whereas arousal appears to depend on a rather focal region of the brainstem, support for awareness may be distributed, with some redundancy, between the AI and pACC. Thus, while AI or pACC lesions alone may be insufficient to significantly impair awareness, disruption of both may be more detrimental (potentially explaining why left hemispheric inhibition with intracarotid amobarbital disrupts consciousness[150]). This possibility may be explored further with simultaneous lesions of both the AI and pACC in experimental animals.

Fourth, resting state functional connectivity, despite its advantages, cannot determine whether functional associations are mediated by mono- or polysynaptic connections, or the directionality of these connections. Previous studies have shown that there are monosynaptic neuronal connections between the PBN and AI[29], and between the AI and ACC[113], but we cannot verify that these connections subserve the functional associations identified here. Ablating these axonal connections in an animal model may lend insight into the importance of these tracts in sustaining consciousness. Regarding directionality, the canonical conception of the ARAS, as projections from the brainstem to the cortex that sustain arousal, predicts that communication occurs predominantly in an ascending (bottom-up) direction. However, it is certainly feasible that communication occurs in a descending (top-down) manner as well, permitting perceptual content to modulate arousal, as in cases of fear or excitement. Indeed, the axonal projections between the PBN and AI are bidirectional[29]. The question of directionally could be assessed with statistical techniques such as the Granger causality analysis[162], which assesses lag between fMRI signal fluctuations of functionally connected regions to infer which region "drives" communication. Such a technique could be applied to fMRI data collected at rest, and again during exposure to a fear stimulus, in order to assess the directionality of communication between the brainstem node, AI, and pACC.

Fifth, while our results implicate the left hemisphere in sustaining consciousness, handedness was difficult to ascertain from the comatose patients, and our rs-fcMRI dataset was obtained from a right-handed cohort. Thus, it is unknown whether lateralization of this network depends upon hemispheric dominance. This could be further explored by identifying and comparing coma-causing brainstem lesions in patients known to be left-handed, as well as by assessing the connectivity of this network in a left-handed cohort.

Sixth, while the correspondence between this network and the VEN distribution is intriguing given the theorized role of VEN's in self-awareness[76,83], these results are only associational. Selectively activating or inhibiting VENs in the AI and pACC would help to clarify their relevance to consciousness and other functions. However, such investigations have been impeded by the selectivity of these neurons to humans and

other mammals not readily subject to experimentation. Recently, though, VENs have been discovered in the AI and ACC of macaque monkeys[163], which are also capable of self-recognition in the mirror test (albeit less robustly than great apes)[164]. As experimentation in macaque monkeys is more common, this discovery may permit more rigorous investigation into the function, physiology and connectivity of these neurons.

Seventh, our analysis in patients with disorders of consciousness revealed that AI-pACC connectivity did not differ between MCS and VS/UWS, as it was strongly diminished in both. Though both conditions involve severe impairments in awareness, MCS patients do demonstrate intermittent signs of awareness, whereas VS/UWS patients demonstrate no such signs. A larger cohort may be necessary to reveal subtle differences in AI-pACC connectivity between these two groups. It should be noted that since this network is profoundly suppressed in both MCS and VS/UWS patients, assessing connectivity of this network in patients with disorders of consciousness may be of limited clinical value, given that distinguishing between these groups remains perhaps the greatest diagnostic challenge in such disorders.

Eighth, while this study identifies possible neuroanatomical substrates for supporting human consciousness, it remains unknown how these regions might operate to maintain consciousness. It is unlikely that these regions, or any particular brain regions, act as solitary “seats of consciousness.” Rather, this network may integrate or coordinate information from across the cortex, and across sensory and cognitive modalities, to produce a unified conscious experience. As the AI and pACC nodes straddle multiple networks, and given prior evidence that these regions act as hubs between networks[143], the network identified here is well positioned to process information in this manner. While the neural processing that produces consciousness will be challenging to investigate, single unit recordings of these network regions in experimental animals may lend insight into how this network responds to sensory stimuli or to activity in lower order sensory-association cortices.

Summary

The brainstem is classically thought to sustain consciousness, but in humans, the precise brainstem region important for sustaining consciousness and its associated network have been poorly defined. First, we investigated the locations of coma-causing brainstem lesions to identify this region. Second, by analyzing spontaneous fluctuations in brain activity, we identified the brain network that communicates with this brainstem region. Third, we evaluated the connectivity of this network in patients with disorders of consciousness. These analyses identified a small region in the left pontine tegmentum critical to sustaining human consciousness. This brainstem region communicates with the left agranular, anterior insula (AI; also known as the frontoinsular cortex) and the pregenual anterior cingulate cortex (pACC), which themselves are highly connected. These cortical sites are implicated in human awareness and match the histologic distribution of von Economo neurons, which are found in the same distribution in a select group of mammals including humans and great apes. Connectivity between the AI and pACC is disrupted in patients with impaired awareness. These results identify a unique human brain network, which may play an important role in integrating arousal and awareness, the two principal components of human consciousness.

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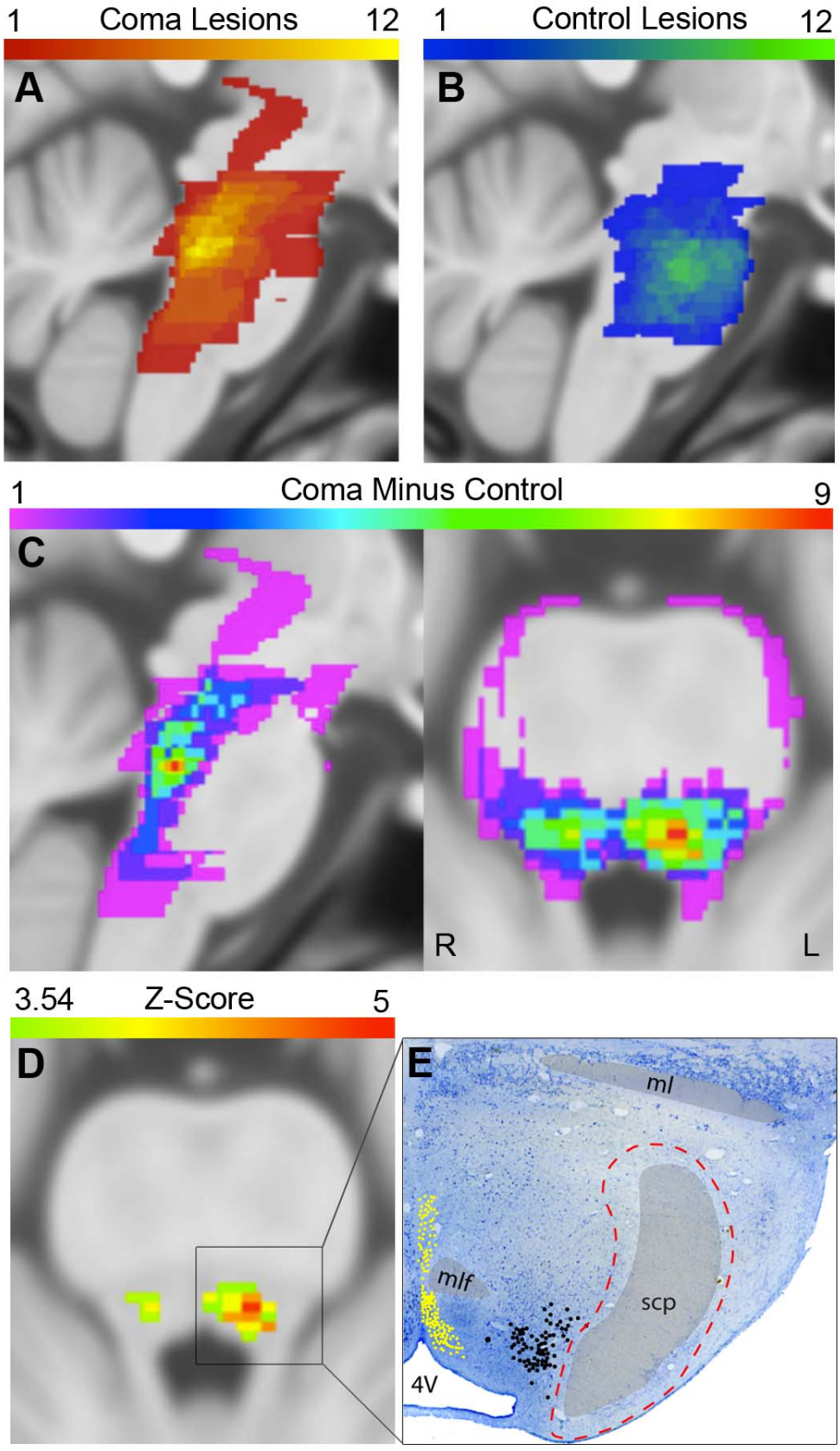
1
Coma Lesions
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A
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Figure 1. **Lesion analysis.** (**A**) Twelve coma lesions exhibited greatest overlap in the upper pontine tegmentum. (**B**) Twenty-four control lesions exhibited greatest overlap in the pontine base. Subtracting the control lesion voxels from the coma lesion voxels (**C**) and voxel-based lesion-symptom mapping ($p < 0.05$, corrected) (**D**) yielded a coma-specific region in the left pontine tegmentum (red). (**E**) Multiple nuclei implicated in arousal surround the coma-specific region, including the dorsal raphe (yellow dots), locus coeruleus (black dots), and parabrachial nucleus (red dashed line). Abbreviations: ml, medial lemniscus; mlf, medial longitudinal fasciculus; scp, superior cerebellar peduncle; 4V, fourth ventricle.

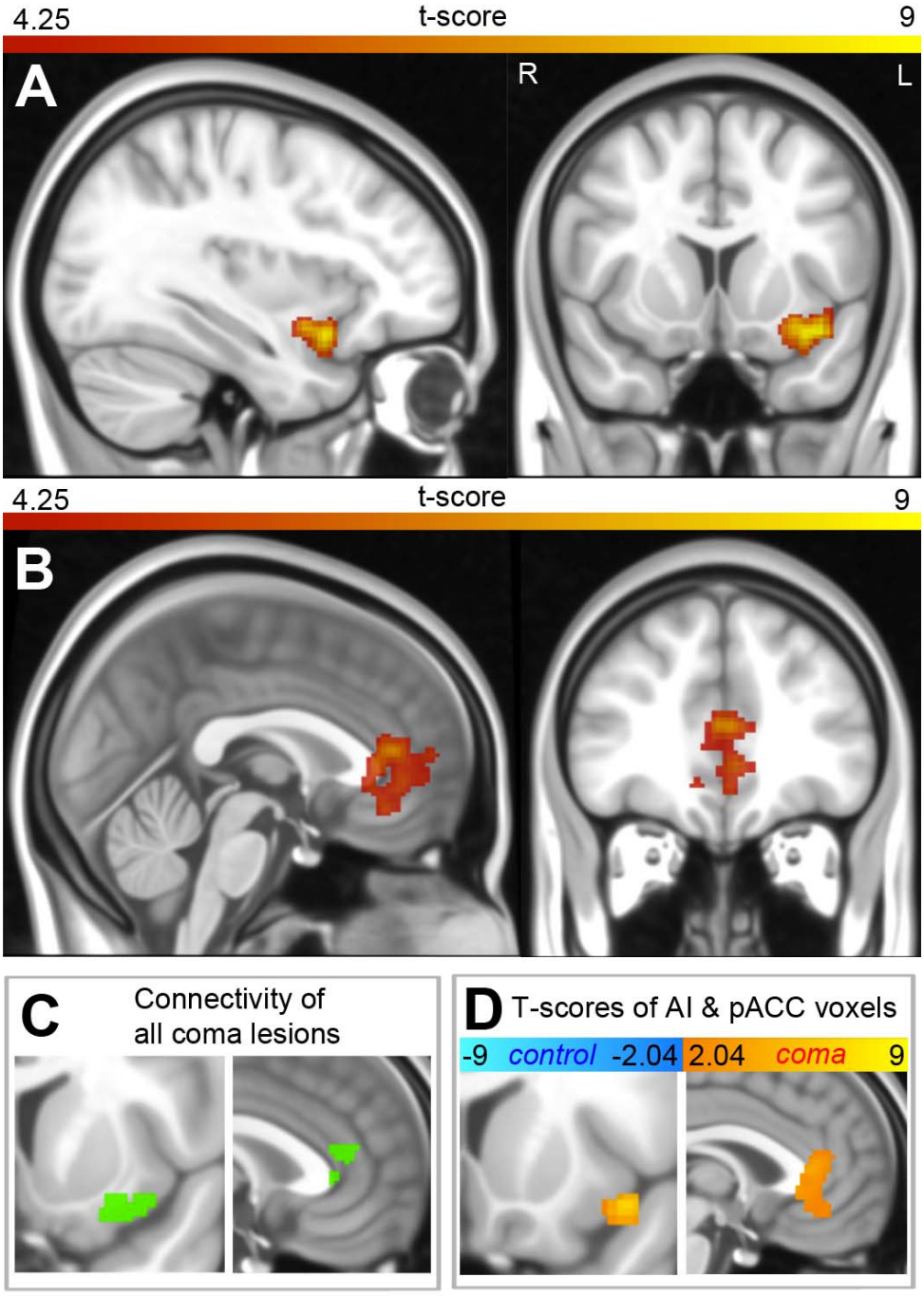

Figure 2. **Network analysis.** The coma-specific region of the brainstem exhibits functional connectivity to clusters in the anterior insula (AI) (**A**) and pregenual anterior cingulate cortex (pACC) (**B**). Voxels within these nodes were functionally connected to all 12 coma lesions (green) (**C**) and were more functionally connected to coma lesions (orange; $p < 0.05$) than control lesions (blue; $p < 0.05$) (**D**).

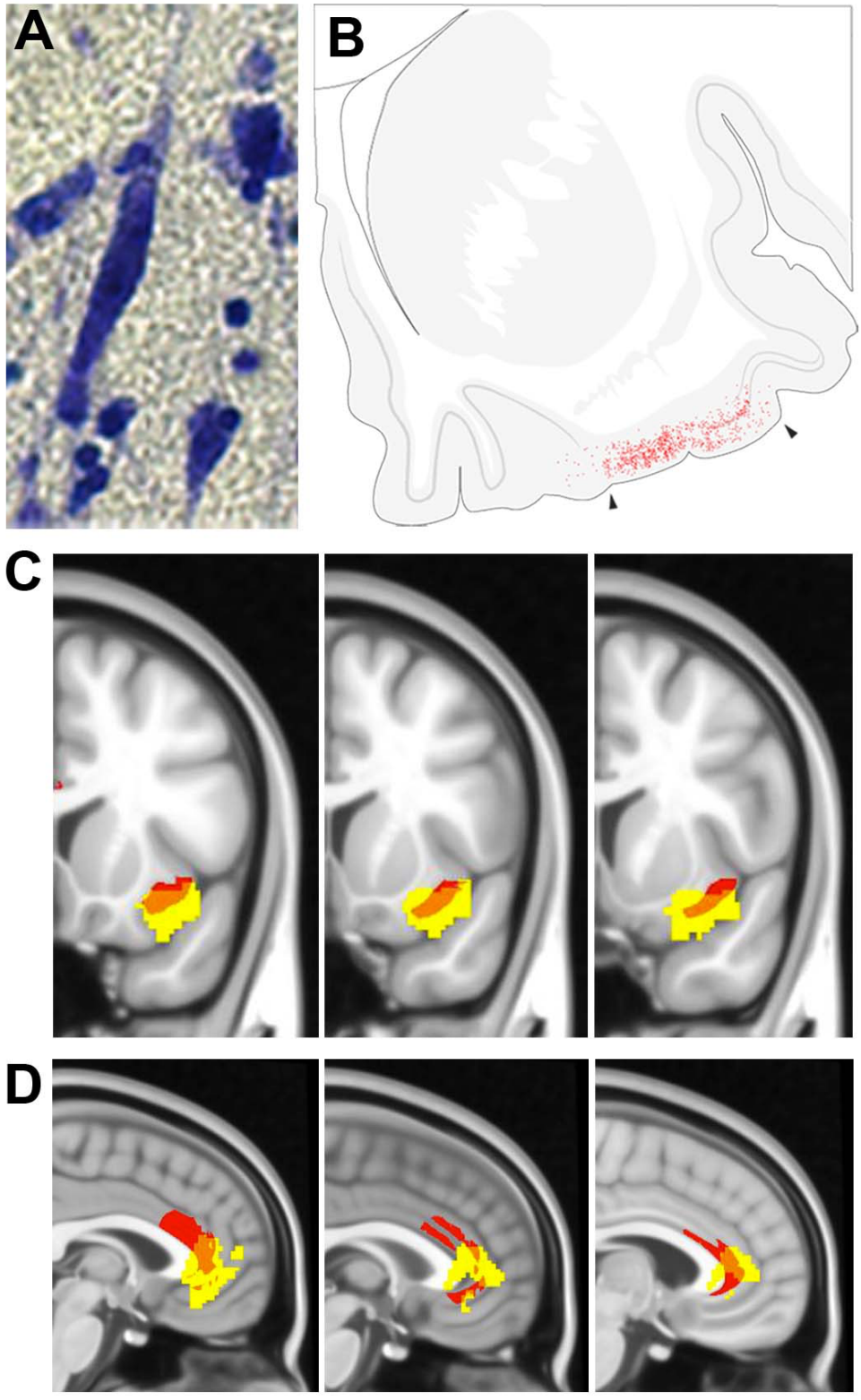
A
B
C
D

Figure 3. Von Economo neuron distribution. Von Economo neurons (VENs), identified by their spindle morphology (**A**), are located in the agranular region (demarcated with black arrows) of the anterior insula, represented by red dots (**B**). The distribution of VENs (red) closely corresponds to the regions of the network nodes (yellow) in the agranular anterior insula (**C**) and pregenual anterior cingulate cortex (**D**).

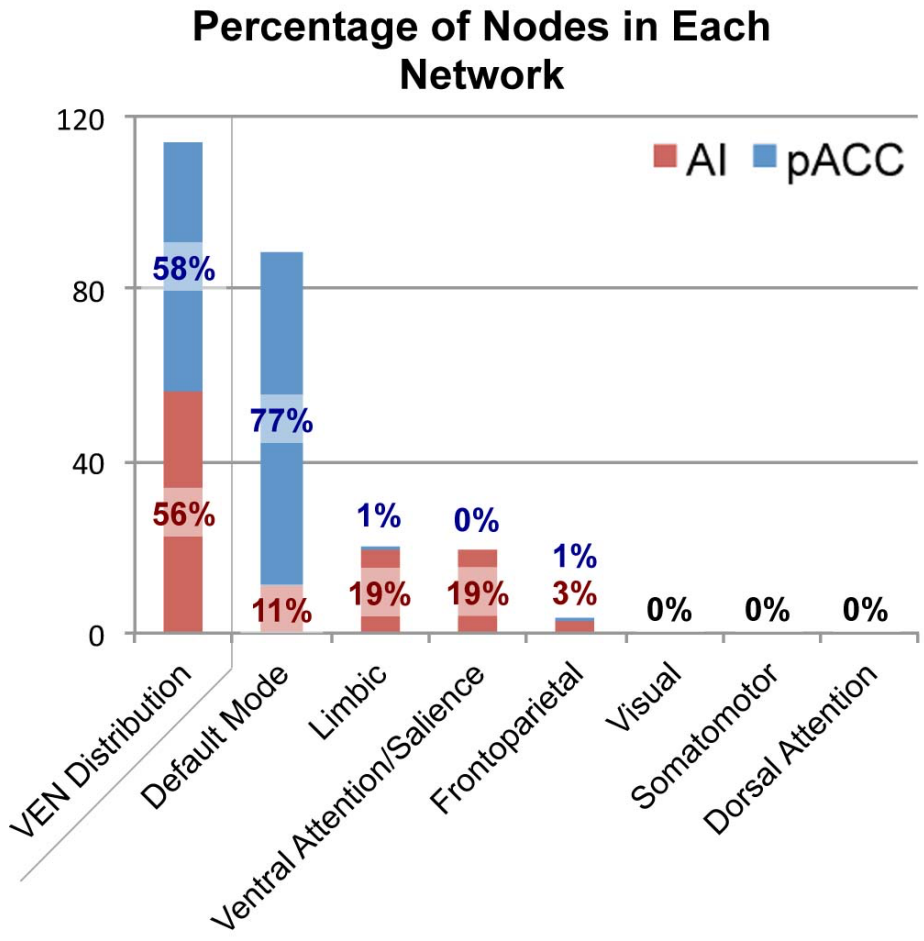

Figure 4. **Network matches von Economo neuron distribution and overlaps several resting state cortical networks.** The anterior insula (AI) and pregenual anterior cingulate (pACC) nodes correspond closely to the distribution of von Economo neurons (VENs), and not to any previously defined resting state network. Rather, the network spans several other resting state networks.

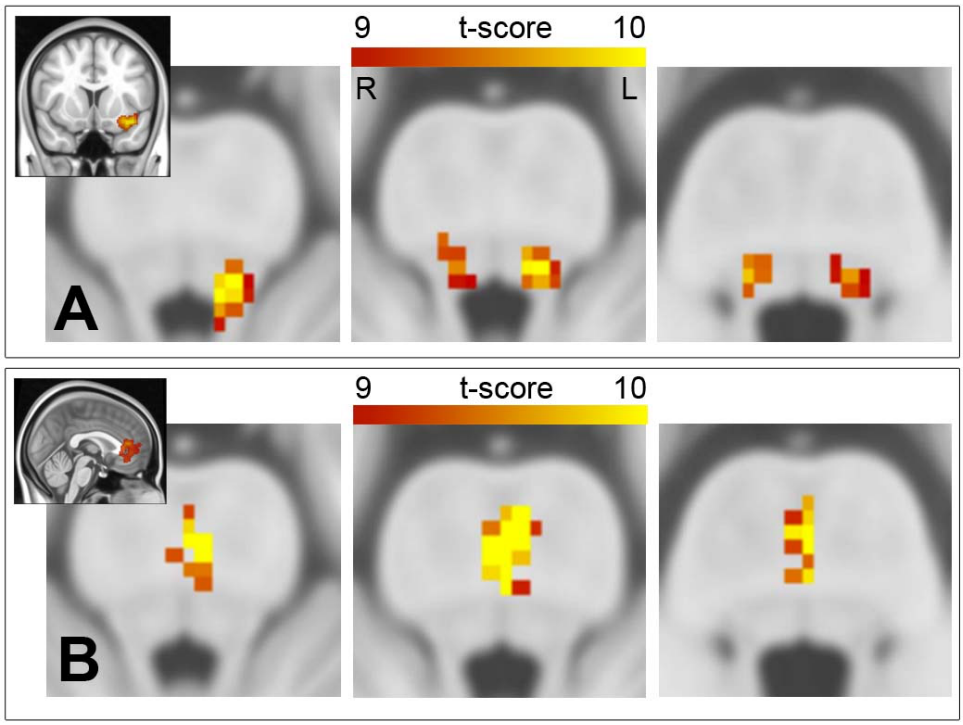

Figure 5. **Brainstem connectivity of cortical nodes.** (**A**) The anterior insula node exhibits functional connectivity to the coma-specific region, and to a lesser extent, the homologous region of the right pontine tegmentum. (**B**) The pregenual anterior cingulate node exhibits functional connectivity only to the pontine base. Images are shown from rostral to caudal.

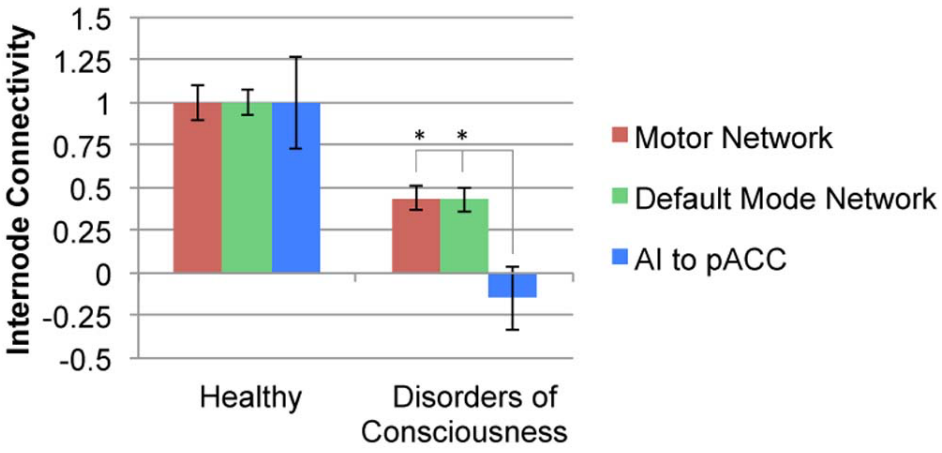

Figure 6. Network connectivity in disorders of consciousness. In patients with disorders of consciousness (minimally conscious state [n=26] or vegetative state/unresponsive wakefulness syndrome [n=19]), connectivity was more diminished between the anterior insula (AI) and pregenual anterior cingulate (pACC) nodes than within the motor or default mode networks ($p < 0.05$). Error bars represent standard error of the mean.


A
coma 1
coma 2
coma 3
coma 4
coma 5
coma 6
coma 7
coma 8
coma 9
coma 10
coma 11
coma 12
control 1
control 2
control 3
control 4
control 5
control 6
control 7
control 8
control 9
control 10
control 11
control 12
control 13
control 14
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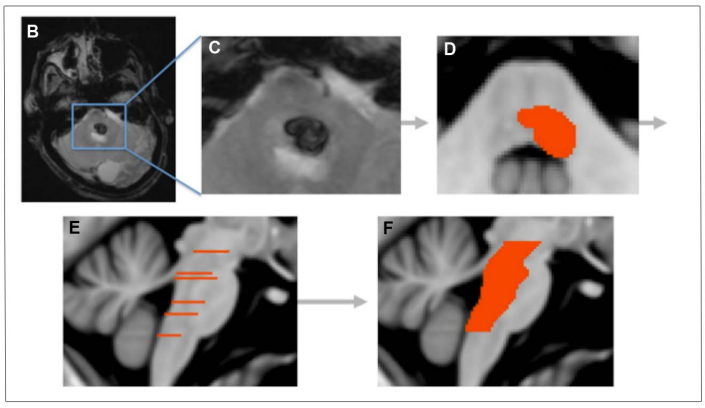
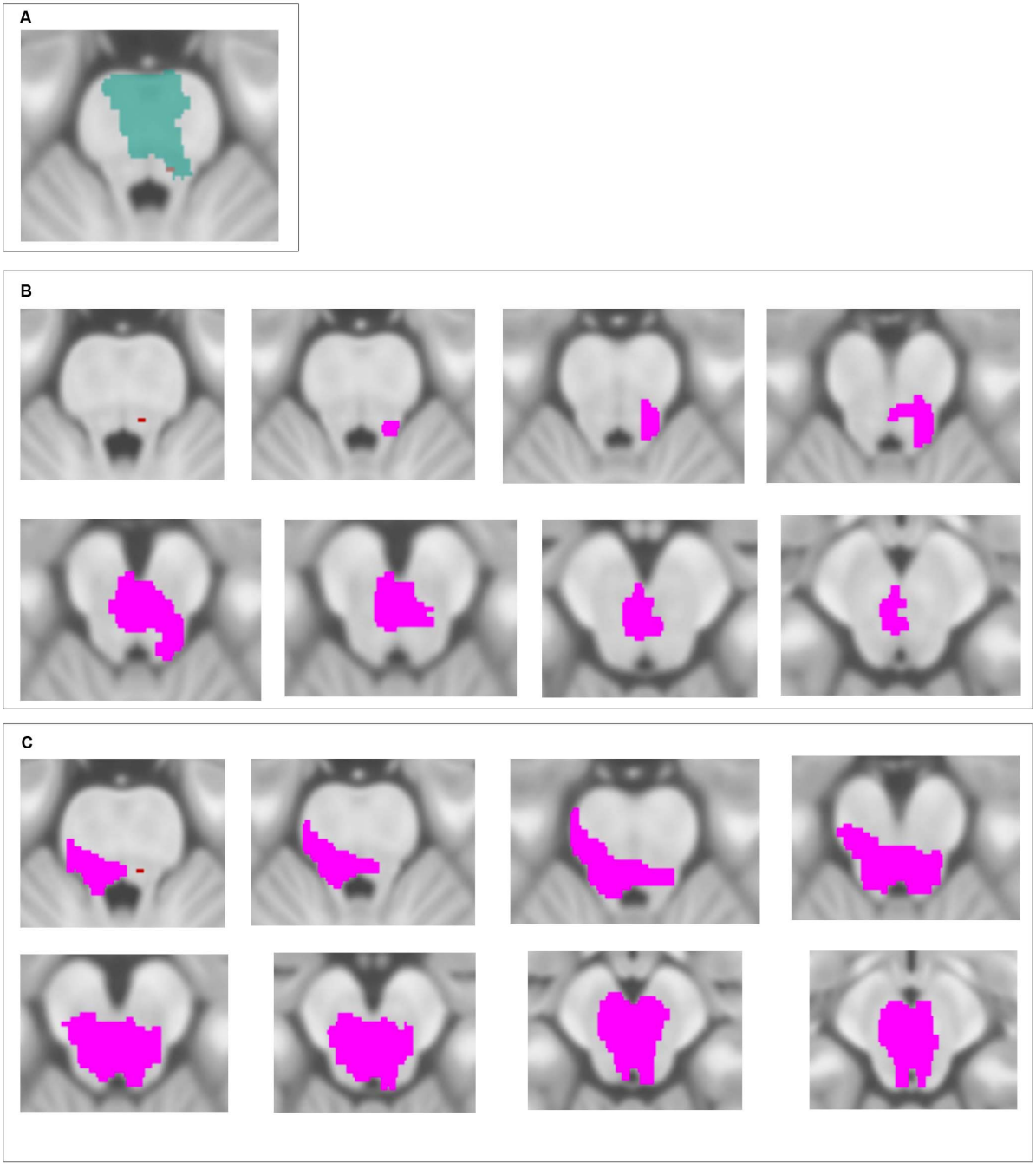
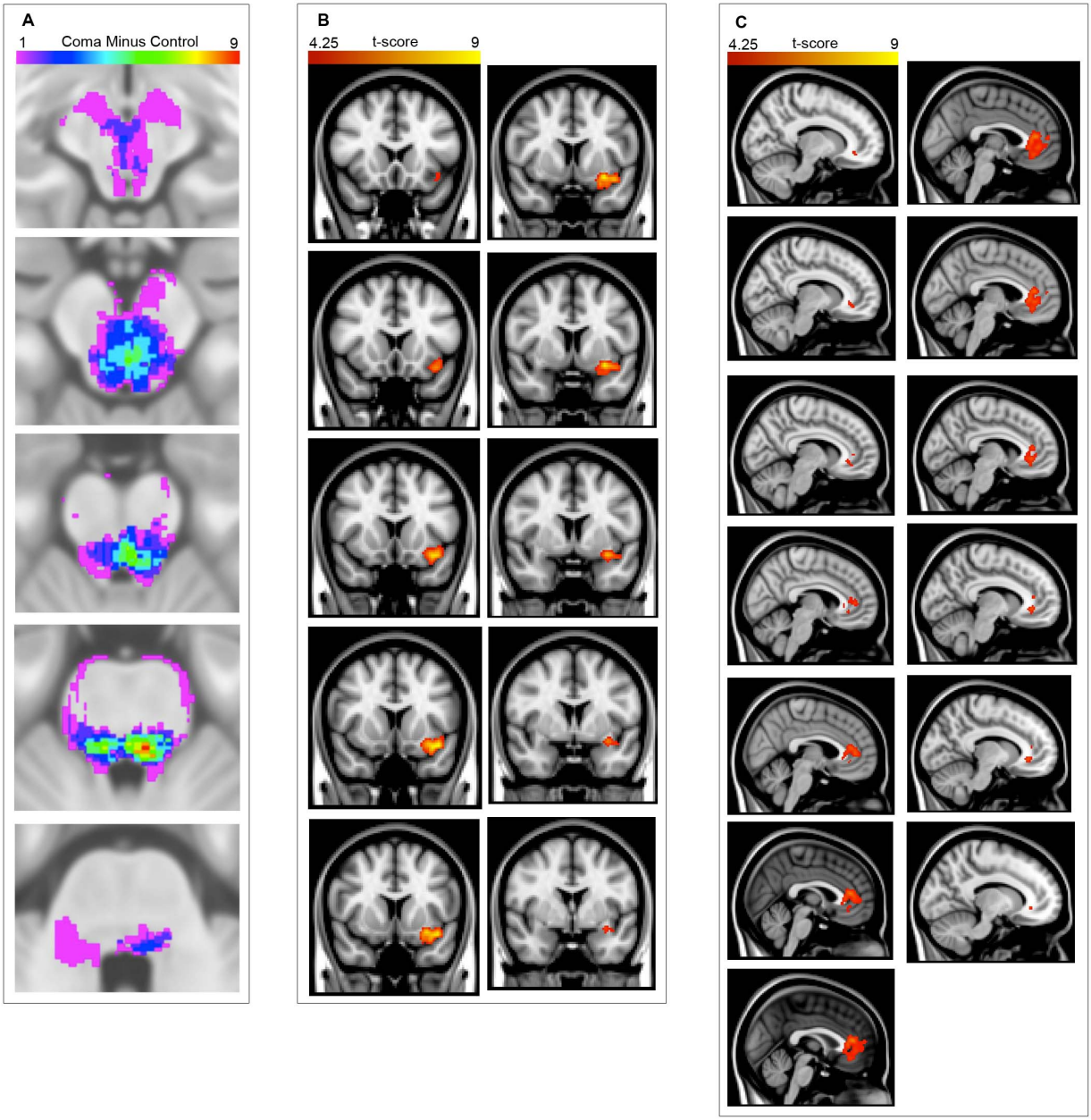
control 23
control 24


B
C
D
E
F

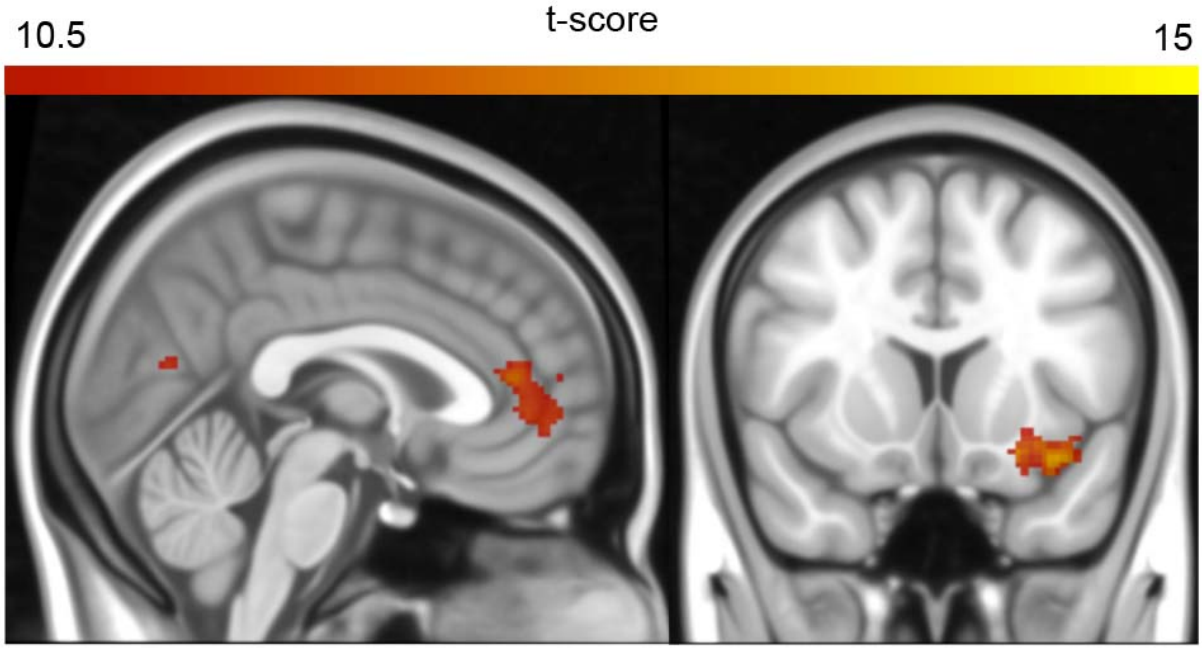
Supplementary Figure 1. Lesions and processing method. (**A**) Sagittal and axial views of each lesion (12 coma lesions, 24 control lesions). For each case (**B**), the brainstem lesion was identified on all available views (**C**). Using anatomical landmarks, each two-dimensional contour of the lesion was reproduced in a comparable view of a template brain (**D**). Once all two-dimensional images of the lesion were reproduced, the lesion was viewed from orthogonal planes (**E**). The lesion edges were connected by the shortest distance and filled in, producing a three-dimensional lesion (**F**).



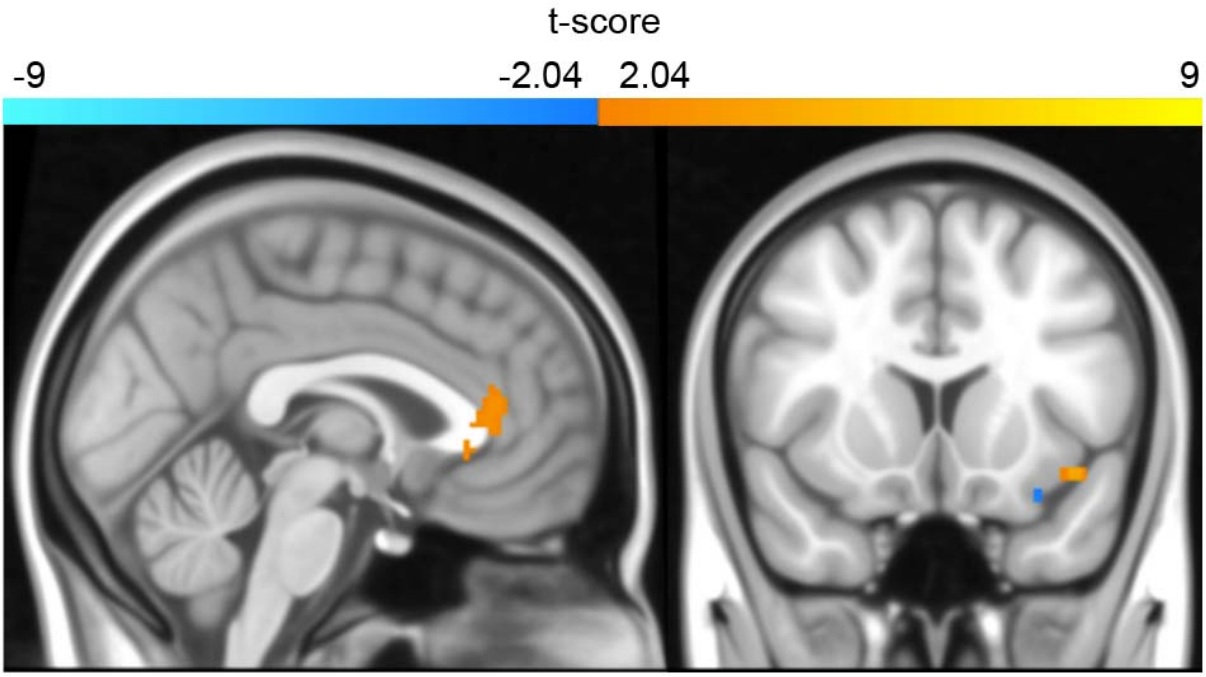
Supplementary Figure 2. Exceptional lesions. The coma-specific region (represented in red) was involved by the periphery of 1 of the 24 control lesions (in green) – Control 4 (**A**) – and not involved by 2 of the 12 coma lesions (in pink) – Coma 10 (**B**) and Coma 12 (**C**). See Supplementary Table 1 for case details. Axial views are shown from caudal to rostral.



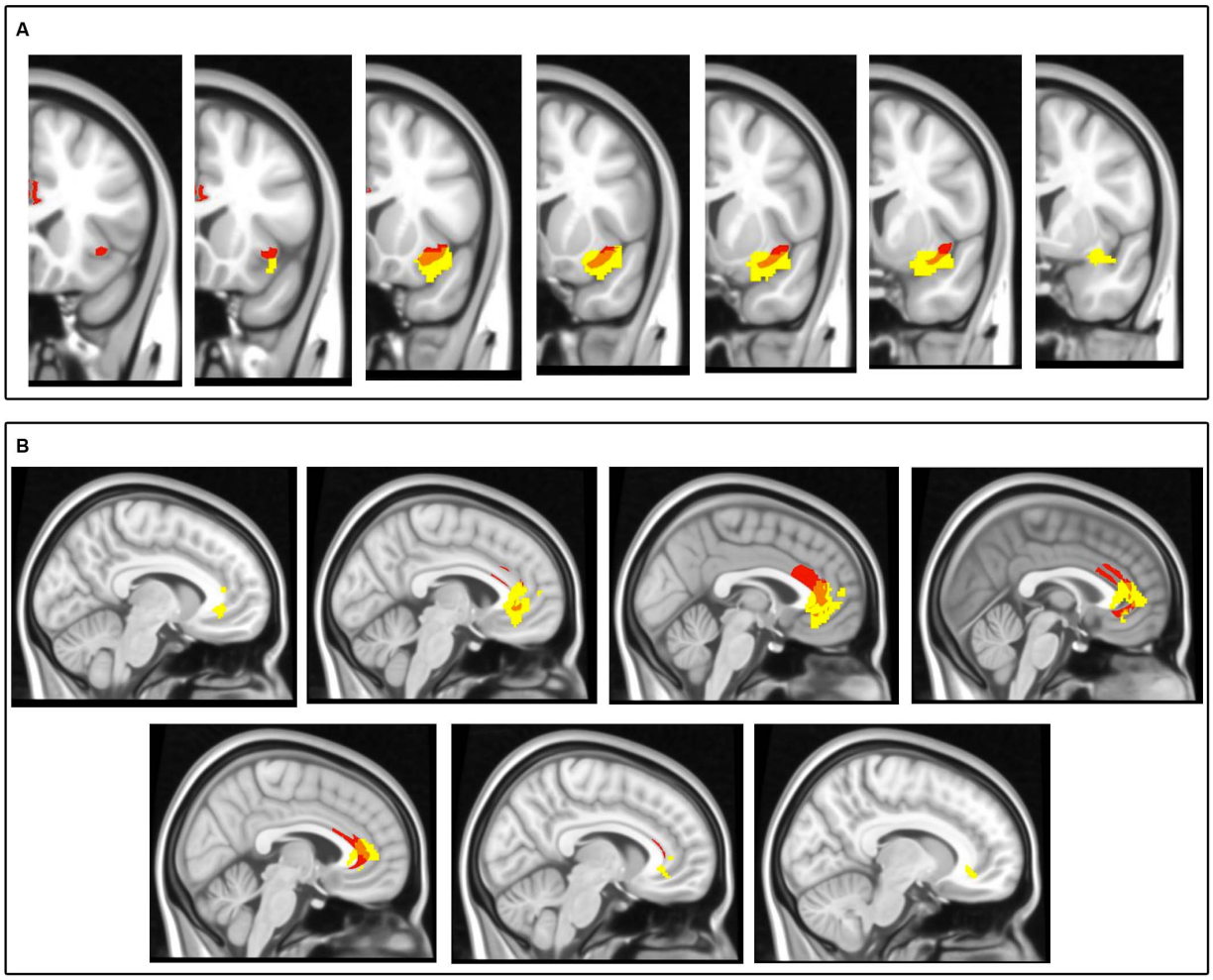
Supplementary Figure 3. Additional views of brainstem node, anterior insula node and anterior cingulate node. (**A**) Axial views of the subtraction analysis, whereby control lesions were subtracted from coma lesions (rostral to caudal). (**B**) Coronal views of anterior insula node (anterior to posterior). (**C**) Sagittal views of pregenual anterior cingulate node (right to left).



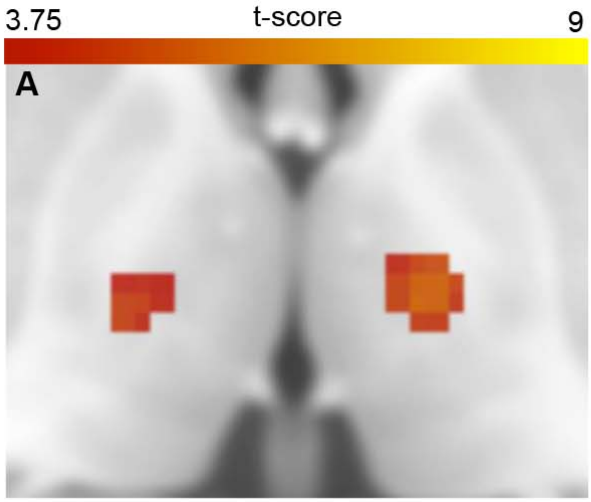
Supplementary Figure 4. Functional connectivity analysis without global signal regression. Functional connectivity of the coma-specific region was repeated without global signal regression, which has been associated with arousal [102]. The anterior insula and pregenual anterior cingulate nodes remain relatively unchanged. See Supplementary Table 2 for additional node details.

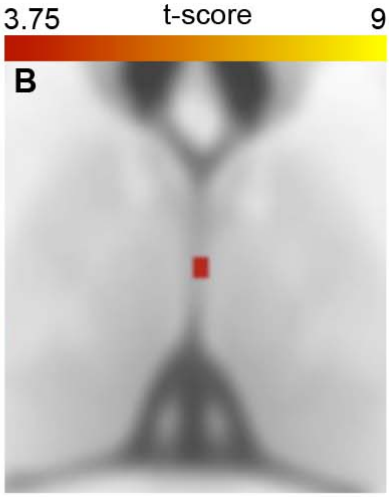


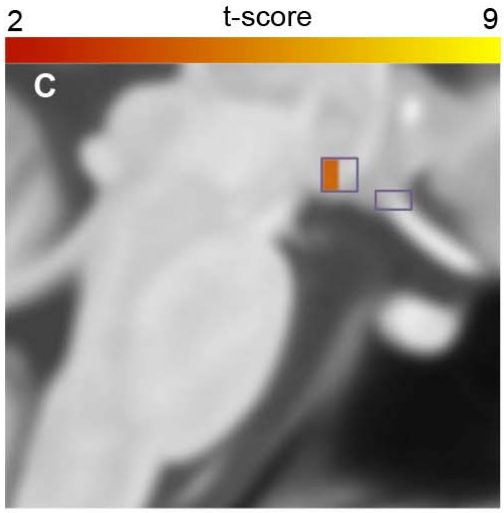
Supplementary Figure 5. Voxel-wise t-test with volume normalization. To ensure that lesion volume did not bias the voxel-wise t-test, each lesion was replaced with a sphere (radius 5 mm) at each lesion's center of gravity. The results of the voxel-wise t-test were masked with the pregenual anterior cingulate and anterior insula nodes. Both nodes remain more strongly functionally connected to coma lesions (in orange; $p < 0.05$) than control lesions (in blue; $p < 0.05$).

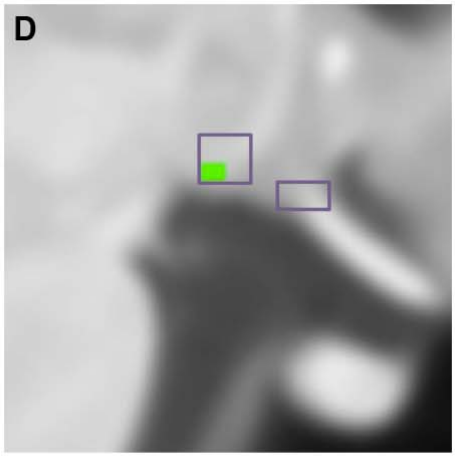

A
B
C
Percentage of Nodes in Each Network
(17 network parcelation; Yeo et al., 2011)
pACC
AI
120
100
80
60
40
20
0
58%
56%
0%
12%
1%
12%
0%
15%
1%
3%
1%
76%
0%
11%
VEN Distribution
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
D
Percentage of Nodes in Each Network
(Smith et al., 2009)
pACC
AI
140
120
100
80
60
40
20
0
58%
56%
51%
0%
3%
0%
13%
95%
21%
VEN Distribution
1 (Visual)
2 (Visual)
3 (Visual)
4 (Default Mode)
5 (Cerebellum)
6 (Sensorimotor)
7 (Auditory)
8 (Executive Control)
9 (Frontoparietal)
10 (Frontoparietal)

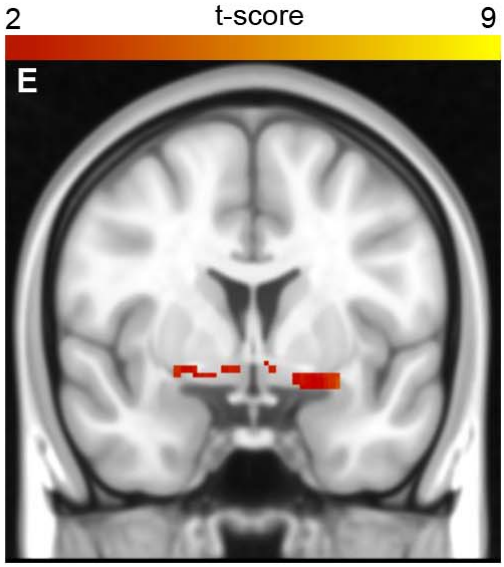
Supplementary Figure 6. Von Economo neurons and network nodes. There is extensive overlap between the Von Economo neuron (VEN) distribution (red) and the network nodes (yellow) of the anterior insula (AI) (**A**, from rostral to caudal) and pregenual anterior cingulate (pACC) (**B**, from left to right). (**C**) A greater percentage of both network nodes is contained within the VEN distribution than within any resting state network.


3.75
t-score
9
A

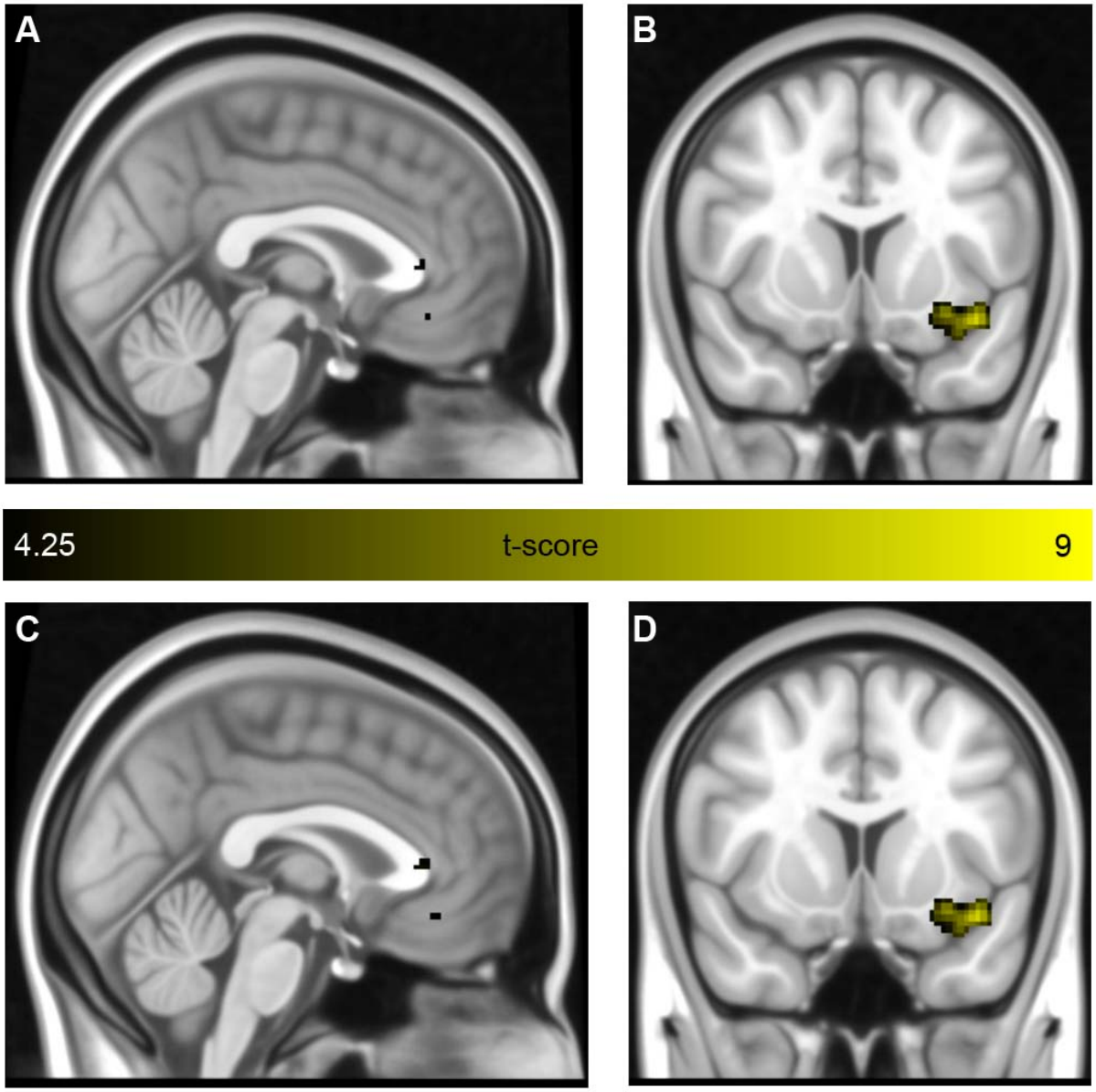

3.75
t-score
9
B


2
t-score
9
C

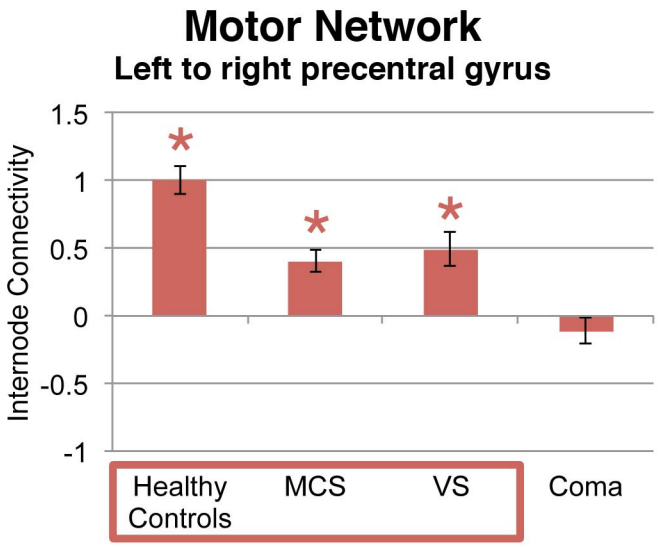

D

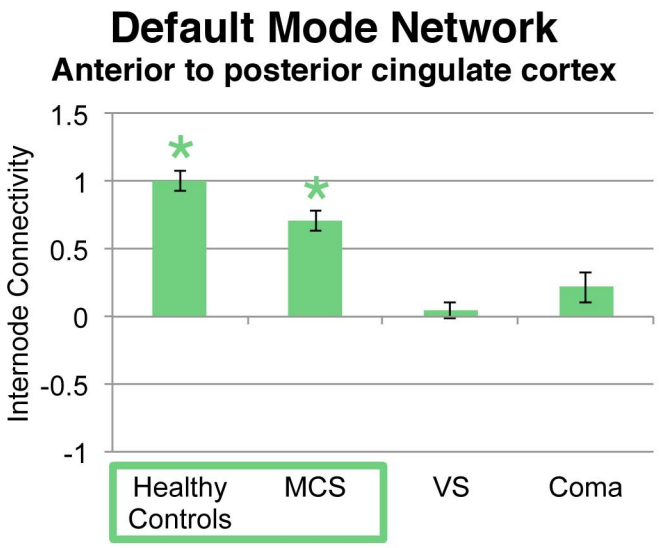

2
t-score
9
E

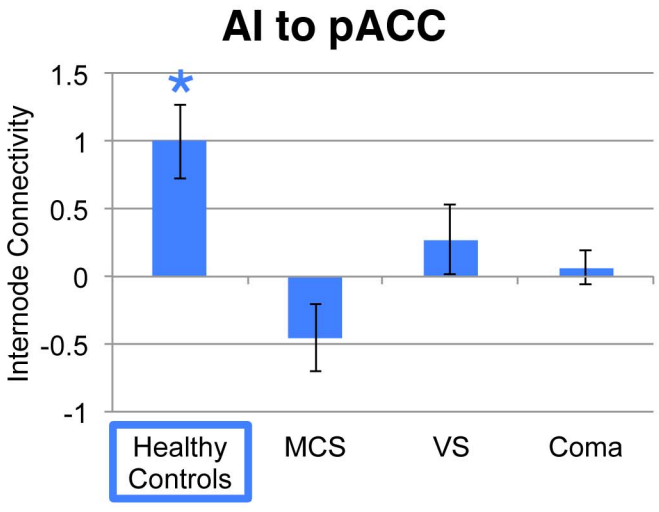
Supplementary Figure 7. Subcortical connectivity. Statistical thresholds were relaxed to specifically investigate connectivity of the brainstem node to the thalamus, hypothalamus, basal forebrain, amygdala and bed nucleus of the stria terminalis. Functionally connected nodes were discovered in the lateral thalamus (**A**), midline thalamus (**B**) posterior hypothalamus (hypothalamus masks outlined in purple) (**C**), and basal forebrain (**E**). (**D**) All 12 coma lesions exhibited connectivity to the posterior hypothalamus (shared region represented in green). There was virtually no connectivity to the amygdala or bed nucleus of the stria terminalis. See Supplementary Table 2 for additional node details.



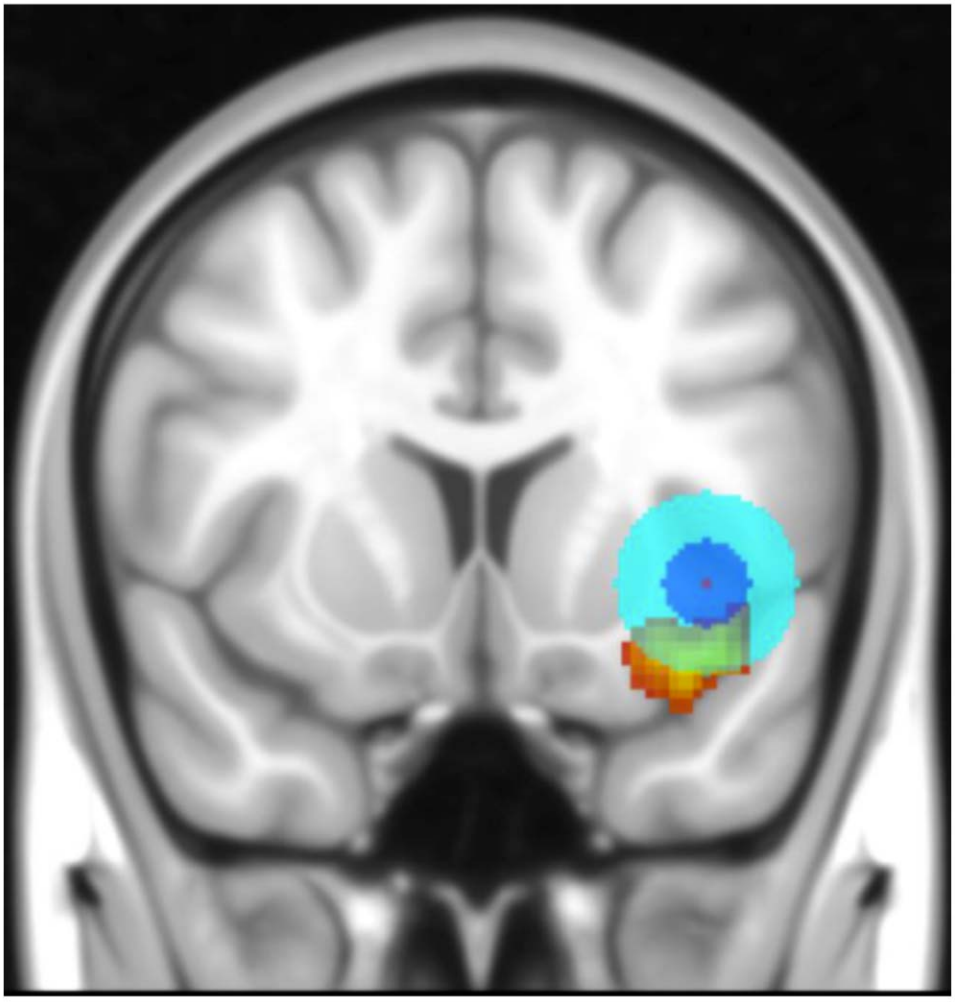
Supplementary Figure 8. Assessing inter-node connectivity with partial correlations. The voxel-wise correlation of the brainstem node to the anterior insula (AI) and pregenual anterior cingulate (pACC), with the other partialled out, was evaluated. With the AI partialled out, little connectivity between the brainstem node and pACC remained (**A**), whereas with the pACC partialled out, connectivity between the brainstem node and AI persisted (**B**), suggesting that the connectivity between the brainstem node and pACC is mediated by the AI. Results were nearly identical after the AI and pACC nodes were binarized, normalizing for voxel values (**C, D,** respectively).


Motor Network
Left to right precentral gyrus
Internode Connectivity
1.5
1
0.5
0
-0.5
-1
*
*
*
Healthy Controls
MCS
VS
Coma


Default Mode Network
Anterior to posterior cingulate cortex
Internode Connectivity
1.5
1
0.5
0
-0.5
-1
*
*
Healthy Controls
MCS
VS
Coma

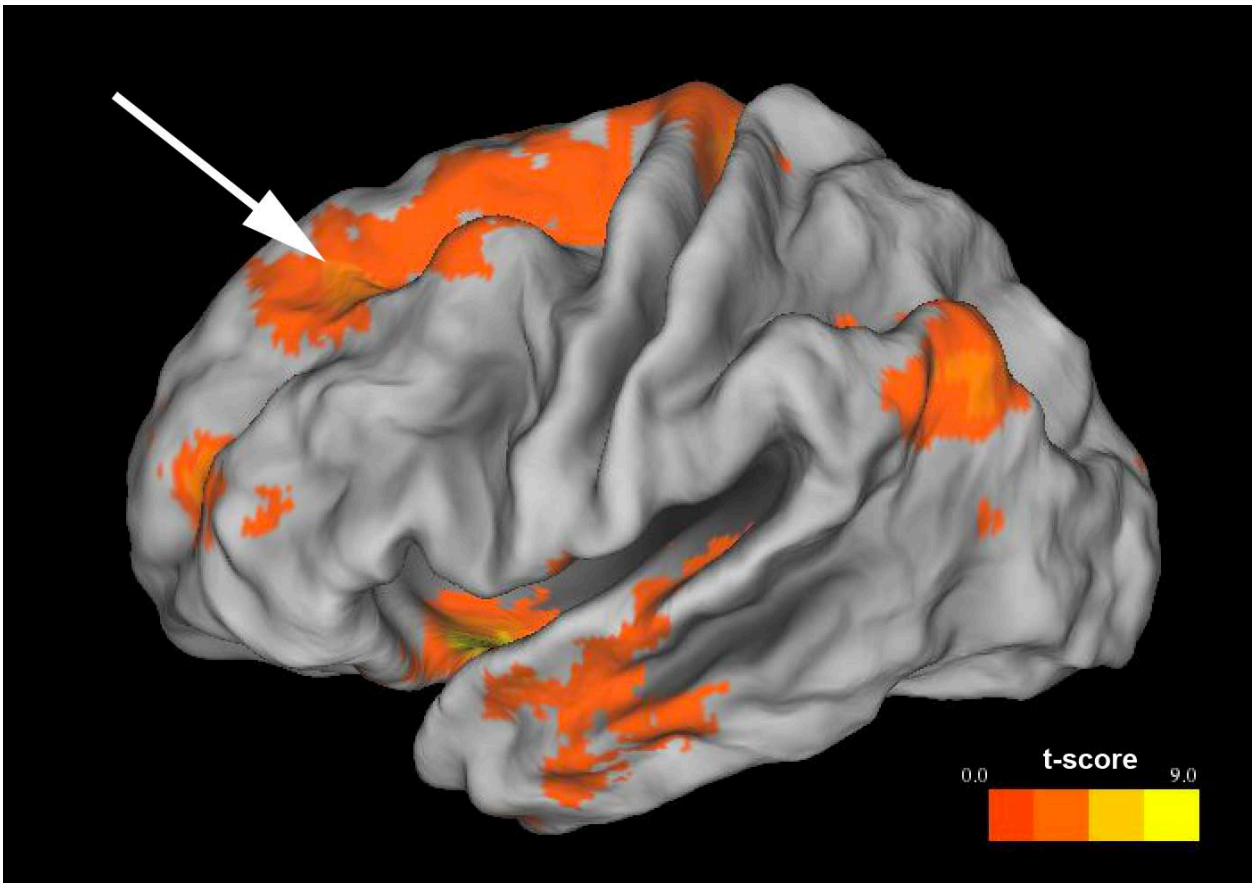

AI to pACC
Internode Connectivity
1.5
1
0.5
0
-0.5
-1
*
Healthy Controls
MCS
VS
Coma

Supplementary Figure 9. Network Connectivity in Disorders of Consciousness. Motor network connectivity was present in all but comatose patients. Default mode network connectivity was present in healthy controls and in the minimally conscious state. Connectivity between the anterior insula and anterior cingulate was present only in healthy controls. MCS = Minimally conscious state; VS = Vegetative state; AI = Anterior insula; pACC = Pregenual anterior cingulate cortex. There were 21 healthy controls, 26 in MCS, 19 in VS/UWS, and 6 in coma. Asterisks represent significant differences from 0 ($p < 0.05$). Error bars represent standard error of the mean.



Supplementary Figure 10. Modeling the electrical disruption of awareness. A previous case report described the disruption of awareness in a human patient after passing 14mA, but not 6mA, of current through a depth electrode placed in the region of the agranular anterior insula[135]. We modeled the effects of each of these current strengths in relation to the anterior insula node identified here (in orange), based on the estimation that the radius of activated neurons increases linearly with current strength (where the radius [mm] equals current [mA] plus 1 for a monopolar electrode)[165]. The depth electrode site was transformed into Montreal Neurological Institute space based on anatomical landmarks (in violet; coordinates: x = -37.5, y = 12, z = -3.5). This site was used as the center of a 15 mm radius sphere (light blue; modeling 14mA) and a 7 mm radius sphere (dark blue; modeling 6mA). In this model, only the 14 mA current,

which was sufficient to disrupt awareness, substantially overlaps the anterior insula node.



Supplementary Figure 11. Functional connectivity of the brainstem node to the dorsolateral prefrontal cortex. Removing statistical thresholds reveals positive functional connectivity between the brainstem node and the left dorsolateral prefrontal cortex (white arrow), a modestly successful target of noninvasive, therapeutic brain stimulation in patients with disorders of consciousness[93]. Figure constructed with Caret software.

Supplementary Table 1. Cases of coma and controls in chronological order.

Case Number	*Age, Sex*	*Location of Lesion*	*Clinical Data*	*Localization Method*	*Citation*
Coma 1	38, F	Pons & midbrain	*Sudden coma following trauma.* See source for detail.	Autopsy	Nyberg-Hansen et al., 1978
Coma 2	75, M	Pons	*Dizziness and numbness followed by coma.* See source for detail.	MRI	Parvizi & Damasio, 2003
Coma 3	61, M	Pons	*Somnolence then coma.* See source for detail.	MRI	Parvizi & Damasio, 2003
Coma 4	73, F	Pons & midbrain	*Low alertness then coma.* See source for detail.	MRI	Parvizi & Damasio, 2003
Coma 5	53, M	Pons & midbrain	*Slurred speech and weakness followed by coma.* See source for detail.	MRI	Parvizi & Damasio, 2003
Coma 6	50, M	Pons	*Sudden coma.* Became unresponsive while walking; hypertensive brainstem hemorrhage identified. Never regained consciousness.	CT	Local Case
Coma 7	79, M	Pons	*Found comatose.* Found comatose in home; brainstem hemorrhage identified. Comatose for	CT	Local Case

			more than a week before emerging to a vegetative state.		
Coma 8	31, M	Pons	*Coma following trauma.* Became comatose following motorcycle accident; brainstem hemorrhage identified. Recovered to full consciousness after ~1 week.	MRI	Local Case
Coma 9	81, M	Pons	*Found comatose.* Found comatose at home; traumatic brain injury identified with brainstem involvement. Never regained consciousness.	MRI	Local Case
Coma 10	45, F	Pons & midbrain	*Coma following trauma.* Pedestrian struck by motor vehicle; traumatic brain injury identified with brainstem involvement. Encephalopathy on EEG. Become responsive weeks later.	MRI	Local Case
Coma 11	51, M	Medulla, pons & midbrain	*Found comatose.* Found comatose at home, with pinpoint pupils; brainstem hemorrhage identified. Regained consciousness ~1 week later.	MRI	Local Case
Coma 12	50, M	Pons,	*Obtundation then coma.*	MRI	Local Case

		midbrain & thalamus	Presented with left-sided hemiparesis and numbness; right vertebral and posterior cerebral artery occlusions identified. Six days later, he became obtunded then comatose; basilar artery occlusion identified. Never regained consciousness.		
Control 1	37, M	Pons & midbrain	*Locked-in syndrome.*	Autopsy	Hawkes, 1974
Control 2	52, F	Pons & midbrain	*Locked-in syndrome.*	Autopsy	Hawkes, 1974
Control 3	49, M	Pons	*Locked-in syndrome.*	Autopsy	Hawkes, 1974
Control 4	35, F	Pons	*Locked-in syndrome.*	Autopsy	Hawkes, 1974
Control 5	74, F	Pons & midbrain	*Locked-in syndrome.*	Autopsy	Dehaene & Dom, 1982
Control 6	34, F	Pons & midbrain	*Weakness, dysarthria.*	MRI	Lu et al., 2011
Control 7	48, M	Pons & midbrain	*Weakness, dysarthria.*	MRI	Lu et al., 2011
Control 8	59, M	Pons & midbrain	*Weakness, dysarthria.*	MRI	Lu et al., 2011
Control 9	62, M	Pons	*Weakness, dizziness.*	MRI	Lu et al., 2011
Control 10	54, F	Pons & midbrain	*Weakness.*	MRI	Lu et al., 2011
Control 11	56, M	Pons	*Weakness.*	MRI	Lu et al., 2011
Control 12	57, M	Pons	*Weakness.*	MRI	Lu et al., 2011
Control	60, M	Pons	*Weakness, dizziness.*	MRI	Lu et al., 2011

13					
Control 14	61, M	Pons	*Weakness, dysarthria.*	MRI	Lu et al., 2011
Control 15	63, M	Pons	*Weakness.*	MRI	Lu et al., 2011
Control 16	68, M	Medulla & pons	*Weakness, dizziness.*	MRI	Lu et al., 2011
Control 17	44, M	Pons & midbrain	*Locked-in syndrome*	MRI	Local Case
Control 18	71, F	Pons	*Awake, no motor deficits*	CT	Local Case
Control 19	64, M	Pons & midbrain	*Awake, no motor deficits*	CT	Local Case
Control 20	67, M	Pons & midbrain	*Awake, no motor deficits*	CT	Local Case
Control 21	59, M	Pons & midbrain	*Nausea, dysarthria, ataxia, weakness*	CT	Local Case
Control 22	71, M	Pons & midbrain	*Weakness, dysarthria, vertigo*	MRI	Local Case
Control 23	62, M	Pons & midbrain	*Confusion, nausea*	MRI	Local Case
Control 24	88, F	Midbrain & thalamus	*Dizziness, weakness*	CT	Local Case

M = male, F = female, MRI = magnetic resonance imaging, CT = computed tomography.

Supplementary Table 2. Peak correlations and centers of clusters.

	Peak Correlation Value	Peak Correlation Voxel			Cluster Center of Gravity		
	t-score	***X***	***Y***	***Z***	***X***	***Y***	***Z***
Coma-Specific Region	N/A	N/A	N/A	N/A	-4.5	-35	-26
Brain-Wide Nodes (Defined by Connectivity of Coma-Specific Region)							
Anterior Insula (AI)	8.96	-40	12	-16	-33.97	9.47	-15.43
Pregnual Anterior Cingulate Cortex (pACC)	6.64	0	38	12	-0.78	37.22	2.47
Brain-Wide Nodes (Defined by Connectivity of Coma-Specific Region), with Global Signal Preserved							
Anterior Insula (AI)	14.1	-30	8	-12	-32	-1.05	-12.6
Precuneus	14.1	-6	-62	12	-1.17	-61.8	11.7
Pregenual Anterior Cingulate Cortex (pACC)	12	-2	38	12	-1.71	44.2	2.12
Thalamic Nodes (Defined by Connectivity of Coma-Specific Region)							

Left Lateral Thalamus	5.58	-12	-16	-2	-11.2	-14.2	-0.67
Right Lateral Thalamus	4.57	18	-18	2	17.4	-16.9	2.17
Midline Thalamus	3.76	0	-14	8	0	-14	8
Basal Forebrain Nodes (Defined by Connectivity of Coma-Specific Region)							
Left Basal Forebrain	4.34	-28	2	-14	-23	1.17	-11.4
Right Basal Forebrain	3.81	28	4	-12	18.4	2.19	-9.88
Medial Basal Forebrain	2.85	-6	2	-8	-5.14	2	-7.14
Brainstem Clusters (Defined by Connectivity of pACC Node)							
Midline Pontine Base	12	0	-24	-28	-0.13	-24.9	-28.1
Brainstem Clusters (Defined by Connectivity of AI Node)							
Left Pontine Tegmentum	10.1	-6	-36	-26	-5.42	-37.4	-26.7
Right Pontine Tegmentum	9.91	8	-36	-32	7.02	-35.9	-29.9